

Science

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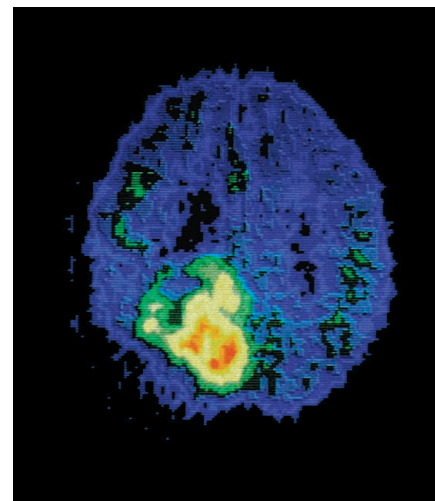
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The porous deposit of candle soot can be used as a template for a super oil- and water-repellent coating. The soot is coated with a thin silica shell to form a replica and is then removed by calcination. The silica is treated with a fluorosilane, yielding a transparent, stable coating. This cheap and easily upscalable approach may inspire the design of anti-fingerprint coatings, which are desirable for touchscreens or glasses. See page 67.

Photo illustration: Bricelyn Strauch and Yana Hammond/Science; candle image: Fotosearch

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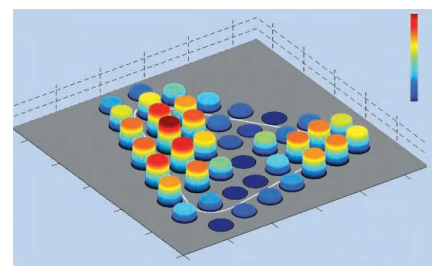
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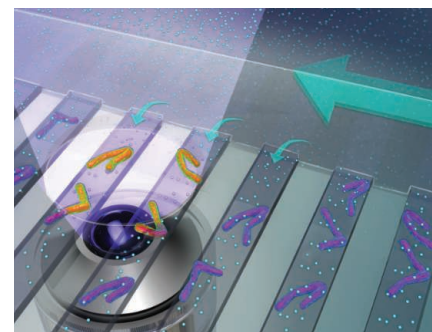
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D. Deng et al.

10.1126/science.1215670

The Crystal Structure of TAL Effector PthXo1 Bound to Its DNA Target

A. Nga-Sze Mak et al.

Structures show how a virulence factor in a plant pathogen recognizes and binds to host DNA.

10.1126/science.1216211

Activation-Induced B Cell Fates Are Selected by Intracellular Stochastic Competition

K. R. Duffy et al.

Cell-fate decisions in activated B lymphocytes are determined by stochastic competition.

10.1126/science.1213230

Centrosome Loss in the Evolution of Planarians

J. Azimzadeh et al.

Analysis of centriole assembly in planaria gives insight into the evolution and function of the centrosome in animal cells.

10.1126/science.1214457

RNA Editing Underlies Temperature Adaptation in K⁺ Channels from Polar Octopuses

S. Garrett and J. J. C. Rosenthal

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10.1126/science.1212795

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Comment on "Nonreciprocal Light Propagation in a Silicon Photonic Circuit"

S. Fan et al.

Full text at www.sciencemag.org/cgi/content/full/335/6064/38-b

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L. Feng et al.

Full text at www.sciencemag.org/cgi/content/full/335/6064/38-c

SCIENCENOW

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Highlights From Our Daily News Coverage

A Surprising Threshold for Seabird Survival Across species, seabird numbers plummet when stocks of prey fish fall below one-third of their maximum size.

<http://scim.ag/SeabirdSurvival>

No Joke: Pigeons Ace a Simple Math Test Observation shows that numerical reasoning is not limited to primates.

http://scim.ag/Pigeons_Math

Elephants Have a Sixth 'Toe'

CT scans help finger a mystery bone in the fat pads of pachyderm feet.

<http://scim.ag/Sixth-Toe>

SCIENCE SIGNALING

www.sciencesignaling.org

The Signal Transduction Knowledge Environment

3 January issue: <http://scim.ag/ss01032012>

EDITORIAL GUIDE: 2011—Signaling Breakthroughs of the Year

E. M. Adler

This year's breakthroughs include structural insights, imaging advances, and new ways to control gene expression.

RESEARCH ARTICLE: Inhibition of PP1 Phosphatase Activity by HBx—A Mechanism for the Activation of Hepatitis B Virus Transcription

D. Cougot et al.

A virus prolongs the activity of a host transcription factor to promote expression of viral genes.

RESEARCH ARTICLE: Incoherent Feedforward Control Governs Adaptation of Activated Ras in a Eukaryotic Chemotaxis Pathway

K. Takeda et al.

Rapid adaptation to changes in chemoattractant concentration involves an incoherent feedforward structure of the underlying signaling network.

LETTERS: Comment and Response on "Load-Induced Modulation of Signal Transduction Networks"—Reconciling Ultrasensitivity with Bifunctionality?

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Complex biochemical and regulatory properties of a bifunctional enzyme mean that its activity cannot be modeled as a simple bifunctional system with distinct and reciprocally regulated states.

SCIENCE TRANSLATIONAL MEDICINE

www.sciencetranslationalmedicine.org

Integrating Medicine and Science

4 January issue: <http://scim.ag/stm010412>

REVIEW: NEW—Network-Enabled Wisdom in Biology, Medicine, and Health Care

E. E. Schadt and J. L. M. Björkegren

Multidimensional networks are required to model human physiology in general and to identify key drivers of pathology in individual patients.

RESEARCH ARTICLE: Vaccine Vectors Derived from a Large Collection of Simian Adenoviruses Induce Potent Cellular Immunity Across Multiple Species

S. Colloca et al.

Simian adenoviruses screened from wild-derived candidates can prime T cell responses in man and may serve as new vaccine vector candidates.

RESEARCH ARTICLE: Novel Adenovirus-Based Vaccines Induce Broad and Sustained T Cell Responses to HCV in Man

E. Barnes et al.

An adenoviral HCV vaccine induces antiviral T cell responses in human volunteers.

FOCUS: Chimp Virus Makes a Savvy Vaccine Vector

M. Houghton

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S. Colloca and A. Colmone

A conversation with Alfredo Nicosia about the discovery of new adenoviruses that will help to make better vaccines to treat human diseases.

RESEARCH ARTICLE: Paracrine Signaling Through MYCN Enhances Tumor-Vascular Interactions in Neuroblastoma

Y. H. Chanthery et al.

PI3K/mTOR inhibitors inhibit angiogenesis by blocking MYCN-dependent paracrine signaling between tumor and endothelial cells.

SCIENCE CAREERS

www.sciencereers.org/career_magazine

Free Career Resources for Scientists

Taken For Granted: Academia's Crooked Money Trail

B. Benderly

Paula Stephan's new book looks beyond rosy press releases to uncover the harsh economic realities of the academic job market.

http://scim.ag/TFG_PaulaStephan

Getting Science to Those Who Need It

G. Koch

A former Silicon Valley entrepreneur found his calling helping biotech-derived therapies reach those who need them most.

<http://scim.ag/SocialBiotech>

Firing Up Tomorrow's Science Stars

S. A. Holgate

Astrophysicist Aude Alapani-Odunlade's true passion is earthbound: helping teachers give their students a solid science education.

<http://scim.ag/FiringUpTomorrow>

SCIENCEPODCAST

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Free Weekly Show

On the 6 January Science Podcast: the parallel evolution of "supersoldier" ants, Ohm's law at the atomic scale, connecting obesity to cancer, and more.

SCIENCEINSIDER

news.sciencemag.org/scienceinsider

Science Policy News and Analysis

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ADVANCING SCIENCE. SERVING SOCIETY

Supersoldier Throwbacks >>

Anomalous traits reflecting those of an ancestor sporadically appear in individuals that normally should not have them. Through their work with the hyperdiverse ant genus *Pheidole*, **Rajakumar *et al.*** (p. 79) suggest that these anomalies represent raw materials for selection to act upon. The ants possess an ancestral developmental potential, to produce “supersoldiers,” that has been retained for over 30 million years for which recurrent induction has facilitated the adaptive and parallel evolution of supersoldiers.



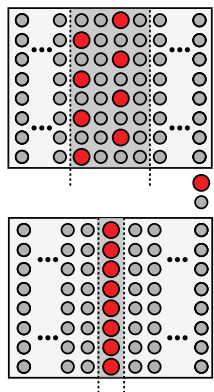
Electrifying Prospects

Greenhouse gas emissions need to be reduced in order to decrease the risk of dangerous climate change, and a commonly advocated intermediate step to decarbonizing our energy production is to cut emissions by 80% by the year 2050.

Williams *et al.* (p. 53, published online 24 November) analyze the infrastructure and technology requirements required to meet this goal in California and conclude that simply using the most technologically advanced types of energy supply now available will not be enough. Instead, transportation and other sectors will need to be converted largely to electrical systems, which would make decarbonized electricity the dominant form of energy supply. Such a transformation will require technologies that are not yet commercialized and intensive public-private and interindustry coordination at every stage of the process.

Wiring Up Silicon Surfaces

One of the challenges in downsizing electronic circuits is maintaining low resistivity of wires,



because shrinking their diameter to near atomic dimensions increases interface effects and can decrease the effectiveness of dopants. **Weber *et al.*** (p. 64; see the Perspective by **Ferry**) created nanowires on a silicon surface with the deposition of phosphorus atoms through decomposition of PH_3 with a scanning tunneling microscope tip. A brief thermal annealing embedded these nanowires,

which varied from 1.5 to 11 nanometers in width, into the silicon surface. Their resistivity

was independent of width, and their current-carrying capability was comparable to that of thicker copper interconnects.

In the Stick of It

If a coating makes a surface nonstick, how do you stick the coating to the surface in the first place? For many nonstick coatings, this involves procedures to ensure good adhesion to the underlying surface through the use of surface roughening or intermediary layers. **Deng *et al.*** (p. 67, published online 1 December; see the cover) found a very simple route using little more than candle soot as a temporary sublayer that is coated with a silica shell and subsequently removed via calcination. Once top-coated with a semifluorinated silane, the resulting material possessed a low surface energy for water and also repelled oils, alcohols, and alkanes. While the coating could be damaged through mechanical wear, the remaining material continued to show superhydrophobic and superoleophobic behavior.

Continental Thermocouple

The patchy presence of billions-of-years-old continental crust indicates a complex coupling between the buoyant forces keeping the lithosphere floating on the mantle and the persistent erosional forces gradually wearing the crust away. Measuring long-term rates of exhumation—the creation of new rock surfaces due to erosion—can reveal how the crust is thermally coupled to the underlying mantle, but techniques to do so have often only been able to resolve a limited temperature range across narrow slices of geologic time. **Blackburn *et al.*** (p. 73) used uranium-lead thermochronology, which is sensitive to the much higher temperatures representative of lower crustal depths, to construct a long-term quantitative model of exhumation and erosion for North America.

Plasmon Probe

When light of certain wavelengths strikes a metal surface, it sets the metal's electrons in motion along trajectories termed “plasmon modes.” **Yurtsever *et al.*** (p. 59; see the Perspective by **Batson**) constructed a type of electron microscope that can probe the electric fields associated with this process by measuring the energy of a separate pulse of electrons that is bounced off the surface immediately (less than trillionths of a second) after the light strikes. The spatial resolution was sufficient to map intensities in distinct regions of a single silver nanoparticle.

Stable Flow

Whole-ocean deep circulation in the Atlantic, the Atlantic Meridional Overturning Circulation (AMOC), transports great quantities of heat from low latitudes to higher ones, which, for example, helps to warm Europe's climate.

Matei *et al.* (p. 76) describe a modeling technique that allows AMOC strength to be predicted for up to 4 years in advance and suggests that AMOC should remain stable until at least 2014.

Musical Chairs

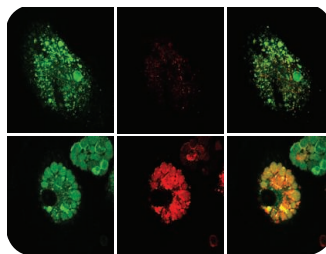
The plant hormone abscisic acid (ABA) helps plants to respond to changes in the environment, such as drought. Physiological responses are initiated when ABA binds to its receptor. In the absence of ABA, downstream kinases are held inactive by phosphatases. **Soon *et al.*** (p. 85, published online 24 November; see the Perspective by **Leung**) now show that both the hormone-receptor complex and the downstream kinase bind to the same site on the phosphatase. Thus, in the presence of hormone, the phosphatase is occupied and unable to interfere with downstream kinase activity.

Going LARGE

Dystroglycan (DG) is a highly glycosylated extracellular matrix (ECM) receptor involved in a variety of physiological processes, including maintenance of skeletal muscle membrane integrity and the structure and function of the central nervous system. The like-acetylglucosaminyltransferase (LARGE) is responsible for posttranslational modifications of alpha-dystroglycan (α -DG) required for its function. Now, **Inamori et al.** (p. 93) demonstrate that LARGE is a bifunctional glycosyltransferase able to transfer xylose and glucuronic acid. These modifications allow α -DG to bind the laminin-G domain-containing ECM ligands: laminin, agrin, and neuexin.

From Nucleoside Recycling to Histiocytosis

Macrophages remove billions of apoptotic cells daily, releasing their nucleic acid material through lysosomal degradation, which allows the resulting nucleosides to be recycled. **Hsu et al.** (p. 89, published online 15 December) found that the nucleoside transporter, equilibrative nucleoside transporter 3 (ENT3), was highly expressed in macrophages and showed that mice deficient in this transporter develop histiocytosis and features of lysosomal storage disease. When exposed to apoptotic cells, macrophages carrying human ENT3 mutations accumulated adenosine and increased their lysosomal pH. These changes contributed to an enhanced signaling through macrophage colony-stimulating factor (M-CSF) receptor and, ultimately, to M-CSF-driven myeloproliferative disease.



Live Fast, Die Faster

Treatment of *Mycobacterium tuberculosis* infections is complicated by the need for a prolonged course of antibiotics to fully eliminate all the bacteria present in an infected individual. Most antibiotics target growth and division machinery; thus, **Aldridge et al.** (p. 100, published online 15 November) postulated that heterogeneity in the growth properties of mycobacterial cells underlies variable antibiotic susceptibility. Cell division in mycobacteria was found to be asymmetrical, with one cell inheriting the growing pole and elongating rapidly, while the other cell elongated more slowly. Over multiple cell divisions, the rapidly growing cells became more susceptible to antibiotic treatment, helping to explain the observed heterogeneity in response to antibiotic therapy.

The Dark Side of Langerhans Cells

Several immune cell populations reside in the skin and are thought to provide a protective barrier against infections and to act as sentinels against malignant transformation. However, studies in mice that lack Langerhans cells, a subset of dendritic cells, have suggested that these cells may actually promote tumorigenesis. Using a mouse model of squamous cell carcinoma, **Modi et al.** (p. 104) now reveal how Langerhans cells may promote the transformation of skin epithelial cells. In response to the carcinogen 7,12-dimethylbenz[α]anthracene (DMBA), Langerhans cells increased their expression of the cytochrome P-450 enzyme CYP1B1, which can metabolize DMBA to the mutagenic DMBA-*trans*-3,4-diol. Thus, besides their functions in regulating the adaptive immune response, Langerhans cells may participate in the metabolism of environmental carcinogens.

Stop, Don't Go There

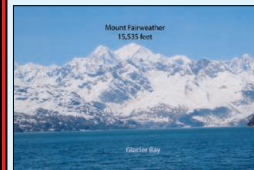
Decision-making in complex brains requires the reaching of a consensus based on information relayed by multiple neurons. A similar situation occurs in the decision-making process of swarming bees, where multiple individuals relay information about suitable hive sites, but a single site is chosen by the swarm. **Seeley et al.** (p. 108, published online 8 December; see the Perspective by **Niven**) show that consensus in this system is reached as information from scouting bees accumulates within the hive. Stop signals were given by scout bees from a particular site to those scout bees signaling from other sites. As the signals accumulate, scouting ceases and the bees prepare to swarm to the site that was best represented among the scouts. When this process was simulated, the results indicated that cross-inhibition among the bees functions similarly to that which occurs among neurons within complex brains. In both cases, such cross-inhibition prevents the overall system from coming to an impasse.

CREDIT: HSU ET AL.

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Bruce Alberts is Editor-in-Chief of *Science*

Voices of the Next Generation

THE START OF 2012 PROVIDES AN OPPORTUNITY TO TAKE STOCK OF WHERE WE HAVE BEEN AND WHERE we are going at *Science*. Internet technology has been rapidly changing the way that scientists access information. This has advantages, allowing new content to be noticed around the globe as soon as it is published. But it also has disadvantages, making it easy for a scientist to receive only preselected information focused on his or her specialty. Because innovative breakthroughs often come from the intersection of disparate ways of thinking, scientists need to continually expose themselves to a broad range of disciplines and approaches. They also must work together as a community to build the strong scientific enterprise needed to create the paradigm-breaking innovations that will be required by an ever-more crowded and resource-limited world. How can we promote the wide-ranging conversations that will be necessary to meet these critical challenges?

We know from surveys that younger scientists primarily read our magazine online, finding specific articles from specialized searches, such as those on Google Scholar or PubMed. As a result, they often miss the valuable, community-building articles in *Science*'s News and Commentary sections, as well as exposure to research in other fields. Many of these young people are already actively engaging in conversations about the issues presented in *Science* on social media sites such as Facebook.* But to bring such conversations to a broader community of scientists, *Science* is providing a prominent space for young scientists in the front half of the print magazine with a new feature called NextGen VOICES. In this issue, we publish the first set of essays from young scientists who responded to our question: "How will the practice of science change in your lifetime?" We received answers from many nations and publish a selection in this issue, some in print (see p. 36) and many more online (www.scim.ag/NextGenResults). We also announce the next question in this series, with essays of 250 words or less due by 17 February: "What is your definition of a successful scientist? How has this definition changed between your mentor's generation and your own?" (see announcement on p. 36 and www.scim.ag/NextGen_2).

The short essays published this week come from young scientists in 18 nations: Austria, China, Denmark, France, Germany, India, Israel, Italy, Netherlands, Norway, Russia, Singapore, South Africa, Spain, Sweden, Turkey, United Kingdom, and United States. The answers are encouraging, inasmuch as they dramatically illustrate the wisdom and energy of the next generation of the world's scientists, along with their strong shared culture. Many of the essays express a greatly increased need for interdisciplinary, collaborative science—calling for new mechanisms and better incentive systems that promote it. All in all, *Science* is very encouraged by the response, and we hope that this new feature will—along with new organizations like the Global Young Academy†—help to empower young scientists to play a larger role in both the scientific community and society.

Other new features at *Science* include two new mechanisms designed to encourage wider participation in the issues published by our magazine. Readers can now post comments on all Commentary, News, and Research content immediately after print publication (see www.comments.sciencemag.org). We also welcome Technical Comments that address core conclusions and methodologies, and will publish those as soon as possible (see www.sciencemag.org/site/feature/contribinfo/prep/gen_info.xhtml). We hope that all of these changes—NextGen VOICES, and the facilitation of both general and technical comments—will allow many more people, and especially young scientists, to participate more effectively in an increasingly global conversation on central issues. Only by heeding the voices of the next generation can we succeed in building a broad-based scientific community that can address the daunting challenges of our times—for in a very real sense, the future is in their hands. —Bruce Alberts

10.1126/science.1218287

*Over 190,000 "likes" on Facebook for *ScienceNow* and 65,000 for *Science*. †B. Alberts, *Science* **332**, 283 (2011).





ECOLOGY

DNA Leaves a Trace

Detecting the presence of populations of rare and threatened animal species is a challenge for conservation planners and managers, especially in habitats like lakes and rivers, where an animal leaves little or no macroscopic trace. Thomsen *et al.* have developed a molecular solution to tracking and quantifying elusive creatures, in the form of DNA obtained from water samples in freshwater habitats in Europe. Their trials used six aquatic species—vertebrate and invertebrate—that occur naturally at low abundance and that are the focus of strict conservation efforts. With samples as small as 15 ml, they were able to detect and quantify populations of each species and to verify experimentally that the DNA indeed represented contemporary occurrence. The technique was then successfully extended to the detection of entire communities of species, using high-throughput sequencing techniques. — AMS

Mol. Ecol. 10.1111/j.1365-294X.2011.05418.x (2011).

ENVIRONMENTAL SCIENCE

Not-So-Quicksilver

In aquatic or subsurface environments, sulfate-reducing bacteria mediate the transformation of inorganic divalent mercury into highly toxic, bioavailable methylmercury (MeHg), but it is unclear how this reaction depends on the phase of the Hg(II). Zhang *et al.* examined the degree to which sulfate-reducing bacteria methylated three forms of Hg(II), representing different size fractions and different states of aging: dissolved Hg(II) ions, 3- to 4-nm-diameter HgS nanoparticles, and >500-nm HgS particles. The bacteria methylated dissolved Hg(II) fastest, but there were also significant differences between nanoparticles and larger particles of HgS that were attributed to size-dependent crystallinity differences and not simply the amount of reactive surface area. Furthermore, aging HgS nanoparticles—which transformed them into larger HgS particles over time—resulted in less MeHg formation as well, suggesting that methylation is related to the intermediate Hg(II) phases present and the crystal growth kinetics. These observations may help explain the varying production rates of MeHg in the environment. — NW

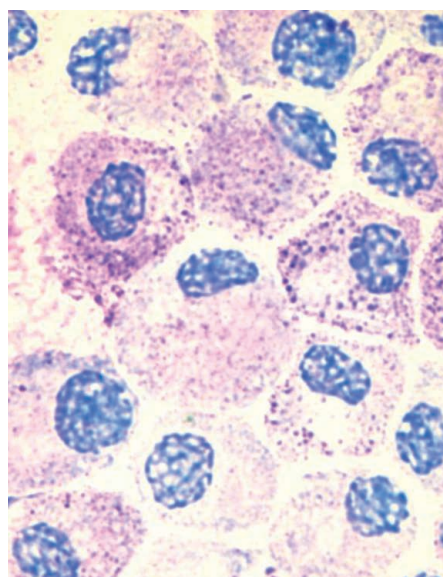
Environ. Sci. Technol. 10.1021/es203181m (2011).

IMMUNOLOGY

Mast Cells Revisited

Although the role of mast cells in allergic disease is well established, they have also been implicated in responses to bacterial infections, autoimmunity, and cancer. Nearly all of these

studies relied on the use of mice containing mutations in the receptor tyrosine kinase Kit, which is important for mast cell development. Although these mice lacked mast cells, Kit is also expressed in other cell lineages and Kit mutant mice suffered from anemia, neutropenia, and impaired lymphocyte development,



among other defects. Feyerabend *et al.* now describe mice (called Cre-Master) with a selective deficiency in mast cells that were generated by the targeted insertion of Cre recombinase into the mast cell carboxypeptidase A3 locus. The insertion of Cre caused deletion of mast cells by genotoxic stress. Cre-Master mice were devoid of mast cells and, as expected, were unable to mount IgE-mediated anaphylactic responses.

In contrast to Kit mutant mice, Cre-Master mice were susceptible to antibody-induced autoimmune arthritis. Thus, the function of mast cells, one of the more enigmatic cells of the immune system, may need to be reevaluated. — KLM

Immunity 35, 832 (2011).

BIOCHEMISTRY

It Takes Three to Copy

The replisome is a complex molecular machine that uses cellular DNA as a template to produce an identical copy. Because of the orientation of the two DNA strands relative to the working direction of the polymerase, the leading strand is synthesized continuously, but the lagging strand is synthesized in segments termed Okazaki fragments. The bacterial replisome was long assumed to contain two polymerases, one each for the lagging strands. Recent studies, however, have provided evidence for three polymerases at *Escherichia coli* replication forks. Why three polymerases would be required remained unclear. Georgescu *et al.* prepared replisomes containing either two or three polymerases and used single-molecule total internal fluorescence microscopy to monitor DNA synthesis. Tripolymerase replisomes were more processive than dipolymerase replisomes, synthesizing products that were nearly twice as long. Differences in DNA synthesis were greater on the lagging than the leading strand, and examination of DNA products showed that the dipolymerase replisome left single-strand gaps, whereas the tripolymerase replisome filled in the lagging strand much more efficiently. On the basis of single-molecule experiments that directly probe

the dynamics of single proteins, it was recently suggested that a new polymerase is used to synthesize each Okazaki fragment during *E. coli* in vivo replication (see Lia *et al.*, Reports, *Science Express*, 22 December 2011). — VV

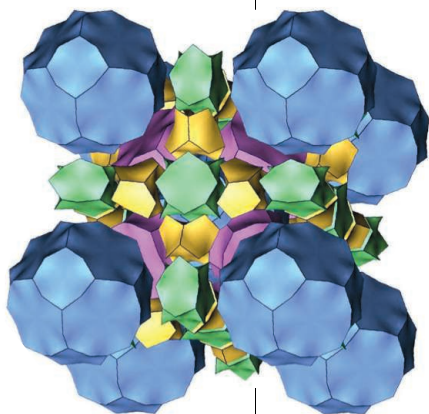
Nat. Struct. Mol. Biol. 10.1038/nsmb.2179 (2011).

CHEMISTRY

Sugary Networks

The burgeoning development of metal organic framework (MOF) solids has primarily relied on the versatile coordination geometries of transition metal ions, which function as lattice nodes bridged in several dimensions by multifunctional ligands. Forgan *et al.* present an in-depth study of a very different class of solids that still fall under the MOF rubric. Alkali cations such as potassium and rubidium replace the traditional transition metals, and the organic components are γ -cyclodextrins—rings of eight covalently tethered sugar molecules better known for one-dimensional encapsulation than for network formation. Body-centered cubic frameworks were crystallographically characterized and proved robust to removal of solvent after their self-assembly in solution. The use of smaller sodium or larger cesium ions led to several different polymorphs, as did divalent strontium, though transition metals proved poor at directing assembly. Among the potassium-linked variants prepared by the authors was a MOF composed entirely of food-grade materials. — JSY

J. Am. Chem. Soc. 10.1021/ja208224f (2011).



NEUROSCIENCE

Fine-Tuning Neuronal Networks

The double-stranded RNA-activated protein kinase (PKR) is widely present in vertebrates, and its activation leads to the phosphorylation of several substrates, the major known cytoplasmic target being the translation initiation factor eIF2 α . PKR is activated in response to a variety of cellular stresses such as viral infection and status epilepticus, and in degenerating neurons in, among others, Huntington's, Parkinson's, Alzheimer's, and Creutzfeldt-Jakob's disease. At present, little is known about its role in normal

neuronal function. Using transgenic mice, electrophysiology, immunohistochemistry, and behavioral analysis, Zhu *et al.* discovered that loss of PKR or pharmacological blockade of PKR activity in mice promoted hyperexcitability in cortical and hippocampal networks and enhanced long-lasting synaptic potentiation and long-term memory. PKR regulated these processes via selective control of GABAergic synaptic transmission mediated by interferon- γ (IFN- γ). These findings thus uncovered a new molecular signaling pathway that regulates network rhythmicity, synaptic plasticity, and memory storage in the adult brain. PKR is activated in various neuropathies and may therefore be a potential therapeutic target. — PRS

Cell 147, 1384 (2011).

HYDROLOGY

A Belgian Water Forecast

Climate change is often discussed in global terms. However, responding to and planning for it requires extending global models to the local level, and assessing impacts on a variety of local resources, such as ecosystems or seasonal water supply, that may have a complex response to the main variables, such as temperature and precipitation. Many uncertainties are associated with such downscaling, regarding how to extend the models and how to assess the local variability in, for instance, precipitation in a future climate. However, approaches are increasingly being developed to consider and explore these uncertainties explicitly, as well as to incorporate ranges of future variability into planning both in the near term and for longer periods. Goderniaux *et al.* illustrate such an approach for assessing the future of groundwater resources in the Geer Basin in Belgium, an important local source of drinking water. In their approach, the authors use the relative change between two global and six regional models, rather than the absolute predicted climates, to build up a stochastic set of future records in key parameters affecting the hydrology of the basin. This allows a probabilistic assessment that can more explicitly represent uncertainties for managers. Overall, they suggest that the climate change signal may dominate normal variability by the later part of this century. Mean groundwater levels are projected to decrease by about 10 m. — BH

Water Resources Res. 47, W12516 (2011).

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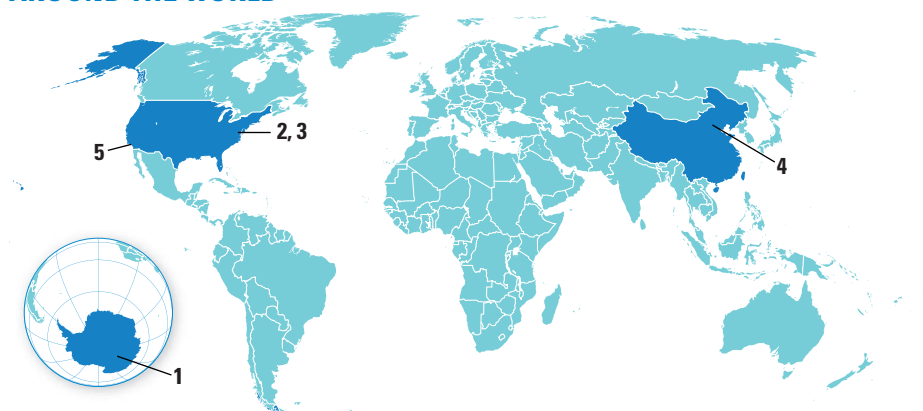
best practices for overcoming skepticism post-Climategate.

One more data point on why you should spend more time at membercentral.aaas.org. There you can enjoy webinars, videos, blogs, discounts, and downloads geared for people who prefer content based on empirical evidence.



membercentral.aaas.org

AROUND THE WORLD



Antarctica 1

Lockheed Gets Contract For Antarctic Support

Lockheed Martin has won a contract worth up to \$2 billion to support U.S. science in Antarctica. The National Science Foundation (NSF) selected the Maryland-based aerospace giant in a competition that lasted more than 2 years longer than expected and



Cold science. McMurdo Station in Antarctica.

featured a record seven bidders, including incumbent Raytheon Polar Services. The agreement, which runs until 2025, extends NSF's history of choosing a new winner each time it rebids the contract, the biggest ever for the agency. The contractor is responsible for providing all of the non-scientific needs of researchers based at NSF's three Antarctic stations. "It's like running a small town," says Erin Tassey, a spokeswoman for Lockheed.

Washington, D.C. 2

NIH Launches Translational Center

As 2011 drew to a close, the National Institutes of Health (NIH) said goodbye to one center and welcomed another. The reorganization became official on 23 December when President Barack Obama signed a 2012 spending bill that includes funding for NIH. The law creates the \$575 million National

Center for Advancing Translational Sciences (NCATS), which will aim to push basic discoveries more quickly to the clinic. And it moves the programs of the National Center for Research Resources to NCATS and other components of NIH. NCATS's acting director is Thomas Insel, director of the National Institute of Mental Health; acting deputy director is Kathy Hudson, NIH deputy director for science, outreach, and policy. A search is underway for a permanent director.

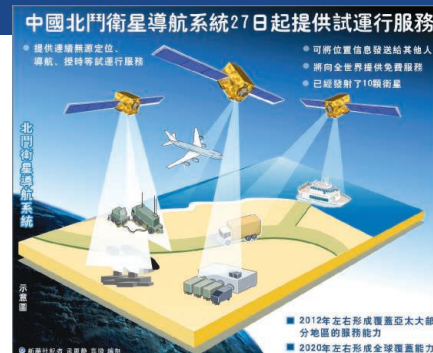
Washington, D.C. 3

Second Paper Pulled on Viral Link to CFS

A *Proceedings of the National Academy of Sciences* (PNAS) paper from 2010 supporting a link between mouse retroviruses called MLV's and chronic fatigue syndrome (CFS) was struck from the scientific record on 27 December—5 days after *Science* fully retracted a controversial 2009 paper suggesting that another mouse-related retrovirus, known as XMRV, is linked to CFS. The retraction took away the only paper left indicating a role for mouse retroviruses in CFS.

The *Science* study, by Judy Mikovits of the Whittemore Peterson Institute for Neuro-Immune Disease in Reno, Nevada, and colleagues, came under fire because other studies couldn't replicate the data. The PNAS paper, whose lead researcher was Shyh-Ching Lo of the Food and Drug Administration, initially seemed to lend support to the findings, although scientists pointed out that it fingered a different group of viruses.

All seven of the PNAS paper's authors signed the retraction, stating that they themselves had been unable to isolate the virus in subsequent work. <http://scim.ag/XMRVpaper2>



In position. China's global positioning network.

Beijing 4

'Big Dipper' Goes Online

China's BeiDou navigation satellite system, also known as "Big Dipper," had begun a trial run, making China the third country in the world to operate such a system, after the United States's Global Positioning System and Russia's GLONASS, the director of the China Satellite Navigation Office, Ran Chengqi, told Chinese reporters at a press conference 27 December. By 2012, with six more satellites added to the current ten, BeiDou plans to offer services to customers in the Asia-Pacific region; by 2020, Beidou plans to have global coverage in operation with 35 satellites.

Meanwhile, two Chinese space science missions moved from the planning stage into an engineering design phase, the Chinese Academy of Sciences announced the same day. One of the new satellites will carry a payload for quantum science experiments, and the other will be looking for evidence of dark matter. Both satellites are part of CAS's effort to boost space science as part of its Innovation 2020 plan (*Science*, 20 May 2011, p. 904).

Los Angeles, California 5

UCLA Professor to Be Charged In Lab Death

The district attorney's office for Los Angeles County filed felony charges on 27 December 2011 against University of California, Los Angeles (UCLA), chemistry professor Patrick Harran, as well as the university itself, for maintaining an unsafe work environment that led to the 2009 death of a 23-year-old research assistant in a lab fire.

On 29 December 2008, Sheharbano "Sheri" Sangji was severely burned while working with highly flammable chemicals without a protective coat in Harran's lab. She died 18 days later (<http://scim.ag/safelabs>).

According to court documents, the district attorney's office holds Harran and UCLA criminally responsible for three

felony counts of violating California's occupational safety regulations, which require that employers ensure that workers receive proper safety training and wear adequate protective equipment. If convicted, Harran could face up to 4.5 years in prison and UCLA could be fined \$1.5 million for each count. Attorneys for UCLA responded that they intend to fight the charges, which they say contradict the findings of California workplace health investigators.

NEWSMAKERS

FBI Investigates Stem Cell Case

A pathology professor is accused of harvesting stem cells from umbilical cords without any oversight and selling them illegally. The Federal Bureau of Investigation (FBI) last week arrested **Vincent Dammai**, 40, of the Medical University of South Carolina in Charleston, along with two other men, in a "fraudulent scheme" to "perform unapproved procedures" with the cells on patients suffering from cancer, autoimmune diseases, and other conditions. The FBI says in its indictment the three reaped more than \$1.5 million from the scheme.

While stem cells derived from cord blood are being studied in dozens of clinical trials, any testing on people must adhere to guidelines set by the U.S. Food and Drug Administration (FDA). That didn't happen here, the FBI alleges; Dammai, for example, was said to have received umbilical cord blood in the mail for the purpose of creating stem cells without FDA oversight. In a statement, the university said Dammai has been placed on administrative leave and "his laboratory and office have been locked and secured."

Cancer Researcher Sued

Craig Thompson, the president of Memorial Sloan-Kettering Cancer Center in New York City, is facing a \$1 billion lawsuit from the cancer center he used to head. The Leonard and Madlyn Abramson Family Cancer Research Institute, part of the Abramson Cancer Center of the University of Pennsylvania, filed a complaint on 13 December alleging that Thompson, who left Abramson in October 2011, hid his involvement with a biotechnology company and deprived Abramson of intellectual property it was owed. Co-defendants in the suit are the company Thompson helped form, Agios Pharmaceuticals of Cambridge, Massachusetts,

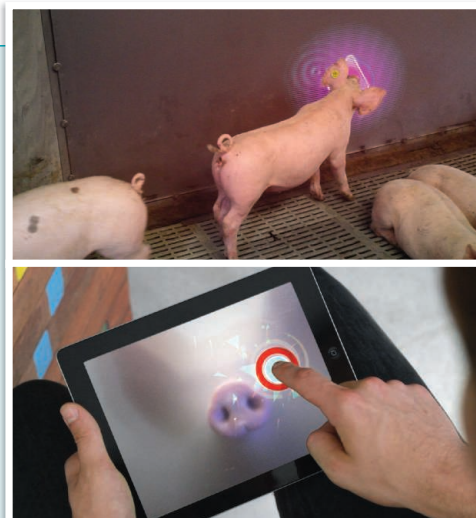
Random Sample

Angry Birds? No, Happy Pigs

Tired of Angry Birds, the game in which you blow little green pigs to bits? If it's up to Dutch designers, you might soon be able to download a more constructive game, designed in collaboration with animal welfare researchers, that lets you play with real, living pigs.

To prevent boredom and aggression in the animals, European regulations require farmers to provide pigs with interesting objects—often a chain with a piece of plastic attached—but nothing seems to hold their interest very long. So designers at the Utrecht School of the Arts came up with the idea for a more interesting game (www.playingwithpigs.nl) that would involve people as well. Via an iPad, a human player would move a bright spot around a big touch screen set up in the barn. Pigs, which apparently love new stimuli, would follow the light with their snout; when the spot moves through a triangle goal, the animal is rewarded with a lightshow while the human racks up points.

The team has yet to actually build a prototype, says designer Kars Alfrink, who admits there are technical challenges—for starters, only one human can play at a time. The main idea, he says, is to get people thinking about their relationship with pigs, which are largely "invisible animals" until they end up on our plates. Co-developer Marc Bracke, who studies animal welfare at Wageningen University and Research Centre, says such a game could be useful for studying cognition and motivation in pigs as well.



and Celgene Corporation of Summit, New Jersey, which has invested in Agios. The institute says damages are "estimated to ultimately exceed \$1 billion."

Through his attorney, Thompson said that "the allegations in this lawsuit are unfounded and without merit." The defendants have until early February to file a response with the court.

<http://scim.ag/CraigThompson>

Three Q's

John Grunsfeld, who this week became head of NASA's \$5 billion science mission directorate, could be a mascot for the space agency: an astronaut who is also an astrophysicist. Grunsfeld has flown on five shuttle flights, most recently on the 2009 mission to service the Hubble Space Telescope. He fills a vacancy left by the departure of Ed Weiler in September last year.



Q: What challenges and opportunities do you see ahead?

The James Webb Space Telescope and the

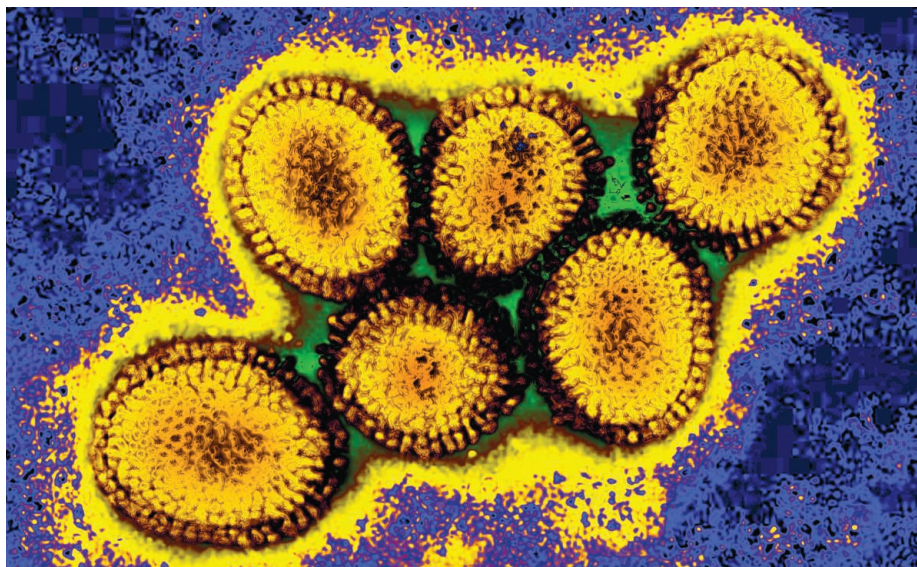
Joint Polar Satellite System are both hard projects that are very important in their fields. We need to show that we have the engineering expertise and the management skills to bring them to fruition. The opportunities are huge. We have satellites like Kepler that are telling us the universe is much richer than we imagined. The opportunities for young researchers to make significant breakthroughs are huge.

Q: Given the budgetary climate, should U.S. researchers scale down their expectations of NASA in the coming decade?

I don't think so. I think the science should drive the expectations. If we allow politics and the budget to determine what science we aspire to do, we'll end up with mediocre science.

Q: Do you suspect you'll be accused by planetary scientists of being prejudiced in favor of astrophysics?

My personal research in recent years has been on exoplanet science. I led the drive that led to the current Mars program. I'm dying to know what's going on on Europa. You could call me a closet planetary scientist. I have also spent a lot of time thinking about heliophysics.



BIOSECURITY

Will Flu Papers Lead to New Research Oversight?

In the fall of 2001, just weeks after the trauma of 11 September, letters laced with powdered anthrax caused death and panic in the United States. Ever since, biological scientists have debated whether, one day, the need to keep sensitive information from aspiring bioterrorists would force them to impose new limits on the academic openness they had long taken for granted.

A decade later, that day appears to have arrived. Just before Christmas, the U.S. government announced that a biosecurity advisory board had asked two research teams to strike key details from papers in press at *Science* and *Nature*. The studies describe how researchers made the deadly H5N1 avian influenza more transmissible between mammals—possibly providing a blueprint on how to set off a flu pandemic. The researchers and the journals agreed, but only if the U.S. government comes up with a system that allows “responsible” scientists to see the deleted information, which public health experts say could be crucial to monitoring H5N1 outbreaks and developing drugs and vaccines.

The unprecedented decision by the National Science Advisory Board for Biosecurity (NSABB) has sparked fierce criticism and strong support. And the episode has implications far beyond a couple of paragraphs in a

pair of papers. Already, senior U.S. officials are scrambling to develop new, tougher oversight procedures for evaluating and possibly blocking potentially risky “dual use” studies before they begin—reviews that critics say the flu experiments should have received. And officials are struggling to devise a workable system for sharing the redacted details with some scientists but not others. “There is no perfect solution” to that problem, says Anthony Fauci, director of the U.S. National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland, which funded the studies. “There is not even a good solution.”

The World Health Organization (WHO), meanwhile, fears that the fallout could damage a 2011 global agreement to share influenza samples that was years in the making. And NSABB chair Paul Keim, a microbial geneticist at Northern Arizona University in Flagstaff, would like to see a voluntary moratorium on publicizing similar studies pending international talks on how to proceed. “This is an Asilomar moment,” Keim

Game changer? Efforts to make H5N1 (left) transmissible in mammals rekindle “dual use” concerns.

says, referring to a landmark 1975 meeting in Asilomar, California, where, after halting their research, scientists drew up safety guidelines for working with recombinant DNA technology.

The studies—led by Ron Fouchier of Erasmus MC in Rotterdam, the Netherlands, and Yoshihiro Kawaoka of the University of Wisconsin, Madison, and the University of Tokyo’s Institute of Medical Science—were designed to see if changes in H5N1’s genetic makeup might make it more capable of jumping from human to human. Kawaoka received an NIAID grant in 2006, while Fouchier’s work was done under a subcontract for Adolfo Garcia-Sastre of Mount Sinai School of Medicine in New York City, who runs an NIAID-funded influenza center.

The studies had not raised eyebrows before. Their dual-use aspects “didn’t hit the radar screen” of the scientists who reviewed the proposals for NIAID, Fauci says, in part because “similar types of research looking at alteration of transmissibility have been going on forever.” University biosafety committees in the United States and the Netherlands also green-lighted the work; such panels typically focus on lab safety, not dual-use aspects.

Little is known about the content of Kawaoka’s study, under review by *Nature*. But Fouchier discussed his work, under review at *Science*, at a September meeting in Malta. His lab initially tried making the virus more transmissible in ferrets—virologists’ preferred animal model—by mutating key genes. That didn’t work, so researchers tried an old method called “passaging”: transferring the virus from ferret to ferret to prod it to adapt. After 10 iterations, Fouchier’s team had a virus that transmitted well thanks to five mutations.

When Fouchier’s paper arrived at *Science*, “it was obvious” that it needed special review, says Editor-in-Chief Bruce Alberts (who did not discuss the paper’s content with *Science*’s reporters). The journal quickly recruited outside specialists, including biosecurity experts who serve on NSABB.

NSABB itself was first



“This is an Asilomar moment.”

—PAUL KEIM,
NORTHERN ARIZONA
UNIVERSITY

Continued on page 22

Continued from page 20

alerted to the studies by NIAID staff late last summer and received copies of the papers in mid-October. The problems were immediately obvious, Keim says. A working group of eight voting and a dozen ex officio members—including White House and NIAID staffers—started discussing the papers in teleconferences. “It was wham-wham-wham, three meetings the first week,” Keim recalls. In November, the working group made its recommendations to the full 25-member NSABB. Several more “very, very serious discussions” followed, says NSABB member Michael Imperiale, a virologist at the University of Michigan, Ann Arbor. “We realized that the stakes were very high for the life sciences.”

NSABB had been there before. In 2005, it had approved a controversial paper about the resurrection of the H1N1 flu strain that caused the 1918 pandemic (*Science*, 7 October 2005, p. 77). “That was a very dangerous virus, too,” Keim says, but for various reasons, the H5N1 work seemed even more problematic. “Slowly, you get to the line where something shouldn’t be communicated,” he says. “These papers exceeded that line in our minds.” In the end, the board was unanimous: Key details had to go.

On 30 November, NSABB members conveyed that message to the scientists and journals. The board, however, has no regulatory authority; it only makes recommendations. And NSABB’s advice was so general that “it took us a while to figure out exactly what they wanted,” Alberts says. By late December, all sides could announce a deal: The journals would publish an incomplete description of the studies—if the government delivered a “transparent plan” for sharing the details.

Kawaoka and Fouchier went along reluctantly. And many of their colleagues say the deal is antithetical to the scientific spirit and unnecessary. “I think it’s an overreaction,” García-Sastre says. Only a small group of highly skilled virologists could make use of the mutations, he says, and the well-known passaging method is “not an ‘Aha!’ idea.” Peter Palese, another flu scientist at Mount Sinai, isn’t convinced that ferret studies can even predict a flu strain’s behavior in humans. Moreover, highly pathogenic H5N1 has been

around for a long time, he writes in an e-mail: “If it would be so easy to make highly transmissible H5N1 viruses, nature would have done it already.”

But a senior flu scientist recruited by NSABB as a special adviser says the panel did the right thing. Robert Webster, 79, of St. Jude Children’s Research Hospital in Memphis, Tennessee, is the unofficial dean of flu science and an expert on H5N1. He believes the studies were justifiable and publishing their main message is essential—which is that H5N1 is a global human threat and “not just a problem for chickens.” At the same time, “I don’t think science should be putting out recipes” for bioterrorists, Webster says.

Now, developing a system to distribute the hidden details is just one of the “very difficult tasks” facing U.S. officials, Fauci says. Fouchier has estimated that perhaps 1000 researchers at 100 institutes should be allowed to see his work, for example. But the government has few legal means of restricting the flow of information other than outright classification, Fauci notes, and “NIH does not do classified research.” Alberts, however, is optimistic that officials will find some “credible method,” perhaps a secure Web site or marked paper copies that could be distributed via embassies.

It won’t work forever, Imperiale predicts. “This information will eventually get out one way or another. We aren’t deluding ourselves.” Even slowing the flow, however, will provide valuable time to develop countermeasures, Imperiale says.

Fauci is also part of a government-wide group that is fiercely haggling over new procedures for spotting “dual-use research of concern” before it starts. NIAID is already reviewing funded projects, he says, to make sure there are no more “surprises.” But what to do about future proposals isn’t clear.

One likely option is expanding a questionnaire-based screening system already in place at a few major universities and for government researchers within the U.S. National Institutes of Health (NIH) and the Centers for Disease Control and Prevention. It asks researchers to check a box if their study might “enhance the harmful consequences of a biological agent or toxin,” such as by increasing transmissibility. The form is then reviewed by an Institutional Biosafety Committee (IBC)—bodies set up

after Asilomar to oversee DNA research—which can recommend changes.

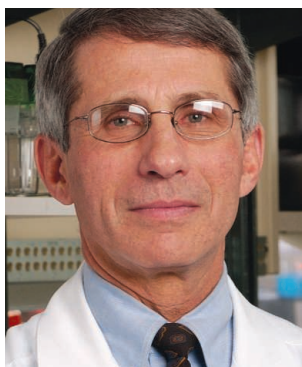
Many researchers and university officials worry that most IBCs—there are some 700 in the United States—don’t have the necessary expertise and that broadly worded questionnaires could hinder research. Studies, however, suggest that few projects would get flagged. When NIH screened 3444 intramural DNA research projects from 2009, for instance, it found just two that merited special review. “There is no excuse for not doing screening,” argues Richard Ebright, a biologist at Rutgers University in Piscataway, New Jersey, and the Howard Hughes Medical Institute. The two flu studies, he says, “would have been clearly identified as problematic much earlier if this process had been followed.”

Clamping down in the United States will have little effect elsewhere. Some biosecurity advocates want a global panel to review studies that could result in a pathogen with pandemic potential; it could be similar to a WHO smallpox research panel that can reject proposed experiments. At the least, an international debate should occur, says Keim, who believes that in the meantime, scientists should neither publish the results of H5N1 transmissibility studies nor present them at meetings. That idea is getting mixed reviews. “I don’t think that’s in the best interest of science,” says virologist Andrew Pekosz of Johns Hopkins University in Baltimore, Maryland. A moratorium could “handicap” young researchers building their careers, he notes.

WHO officials, meanwhile, said in a 30 December statement that they are “deeply concerned about the potential negative consequences” of the controversy on global efforts to share information about H5N1. After the virus started spreading rapidly in 2003, some nations complained about WHO’s system for sharing viral samples and research benefits: Indonesia even suspended participation. To ease tensions, WHO brokered a new agreement, released in May 2011. Redacting the flu papers could violate that agreement—a particularly sensitive issue because WHO says it provided the viral samples used by Fouchier and Kawaoka.

Meanwhile, some scientists worry that the focus on bioterrorism has diverted attention from a more likely risk: a lab accident that could unintentionally release a newly created viral strain. Sloppy lab practices in Asia, they note, triggered three minioutbreaks of the virus that causes SARS. Escape of an engineered H5N1 virus, Ebright says, would “really raise the question of whether there are some studies that aren’t worth the risk.”

—MARTIN ENSERINK AND DAVID MALAKOFF



“There is no perfect solution. There is not even a good solution.”

—ANTHONY FAUCI, NIAID

In the Eye of the Storm, Two Rivals, Two Strategies

The two influenza researchers whose work has triggered a far-reaching debate on the limits of scientific freedom could hardly have handled their publicity more differently.

Ron Fouchier, who has a paper under review in *Science*, welcomed reporters to his lab at Erasmus MC in Rotterdam last month, donned a lab coat for the cameras, and helped the center's spokespeople prepare statements in Dutch and English on why he created what he believes to be a virus with the potential to cause a pandemic and why the full details of the study should be published. He landed on the front page of *The New York Times*, was attacked by bloggers, and news anchors mangled his name. (It's FOOSHAY.) But Fouchier had a point to make: He had nothing to hide.

Yoshihiro Kawaoka, meanwhile, appeared to be in hiding. He didn't, as reporters say, respond to multiple requests for comment, including for this story; it was hard to know whether he was at his lab at the University of Wisconsin (UW), Madison, where his H5N1 study now under review at *Nature* was done, or at the University of Tokyo's Institute of Medical Science, where he has an appointment as well. As a result, he literally managed to stay out of the picture. News stories focused on Fouchier; many didn't even mention Kawaoka.

Some scientists say that may reflect different personalities and backgrounds. Fouchier is known to be open and direct—to the point of bluntness—"a wonderful Dutch quality," says mathematician Derek Smith of the University of Cambridge in the United Kingdom, a close friend and co-author of many of Fouchier's papers. Kawaoka's silence bespeaks his origins, says his former mentor, Robert Webster of St. Jude Children's Research Hospital in Memphis, Tennessee. "In Japanese culture, saying nothing is often a more powerful statement," Webster says. "You smile and take it."

Others aren't so sure. "I think Kawaoka has lived in the U.S. long enough to have lost his Japanese roots," says virologist Andrew Pekosz of Johns Hopkins University in Baltimore, Maryland, "and he's not shy in public." Daryl Buss, the dean of UW Madison's veterinary school, says Kawaoka doesn't want to imperil his paper in *Nature* by discussing it. But he concedes that, as Fouchier did, Kawaoka could discuss the background of the work or the policy questions without jeopardizing publication. "He has chosen not to do that," Buss says.

Their media strategies aside, most scientists say they're more struck by the commonalities between Kawaoka, 56, and Fouchier, 45. Both are "truly top-notch, fantastic scientists," Pekosz says. They're also competitors and rivals, he says—"in a healthy way." Both have taken a strong interest

in avian influenza, and at meetings, they often speak in the same session. "Their labs both run the gamut from amino acids to transmission between humans, which is fantastic," Pekosz says. "It's no accident" that both came up with major new findings on H5N1 transmissibility, Smith adds.

Extremely hard workers, both have built up impressive publication records. Fouchier's name is on almost a dozen papers and reviews in *Science* and a few in *Nature*; for Kawaoka, it's the other way around. For both, the controversy swirling around their recent work has made the past few months a particularly intense period. "All these different opinions create a lot of stress," says Fouchier's collaborator, Adolfo García-Sastre of Mount Sinai School of Medicine in New York City. Kawaoka "is wiped out, I gather," Webster says.

Kawaoka, who grew up in Kobe and trained at Hokkaido University, learned the ropes at Webster's lab in Memphis, where he worked for 14 years. ("My best postdoc ever," Webster says.) He started his own lab in Madison in 1997 and soon became recognized as a scientific superstar; the university invested millions in a new lab and additional staff to retain him when he received a grandiose offer from the University of Pittsburgh in 2004. Kawaoka frequently travels to Tokyo to keep his group there running and lure the brightest Japanese students to Madison. Rather than wearing him out, the 16-hour trips across the dateline "seem to energize him," Buss says.

Fouchier, meanwhile, has built his career at Erasmus MC under the wings of Albert Osterhaus, a prolific virologist who has worked on a plethora of viruses. So far, Osterhaus has been the lab's omnipresent spokesperson; the current controversy marks the first time Fouchier has stepped into the global limelight.

Both Kawaoka and Fouchier have a long history with H5N1. In 1997, Osterhaus's lab discovered that a 3-year-old boy in Hong Kong who had died of pneumonia was infected with the virus—the first recorded human infection with the avian strain, which was wreaking havoc in Hong Kong poultry at the time. Kawaoka spent several months in Hong Kong as part of a team studying the outbreak, which eventually killed 18 people. Fouchier, who had been mostly working on HIV, switched to influenza soon after.

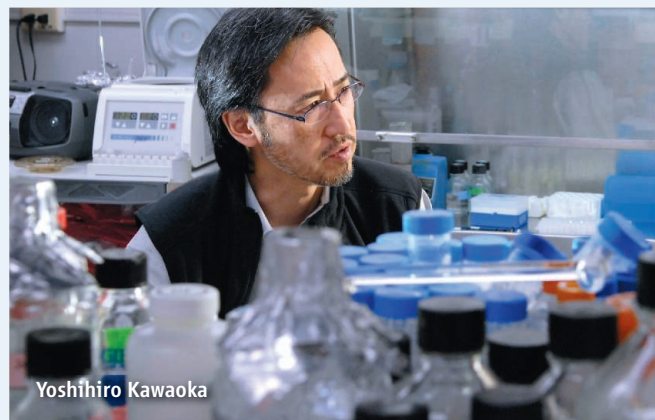
Since then, H5N1 has infected birds in dozens of countries and killed more than 500 people. And Fouchier and Kawaoka have been fascinated by what Pekosz says is "the most pressing question" about the virus: Could it become transmissible between humans—in other words, does it have the potential to become pandemic—and if so, what does it take?

Fifteen years after that first encounter, their unpublished studies may begin to answer those questions.

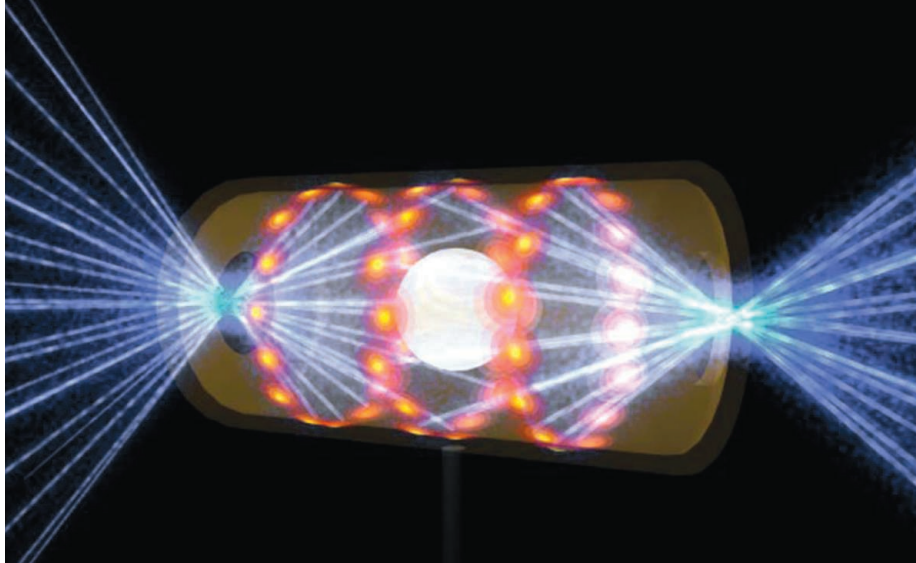
—MARTIN ENSERINK



Ron Fouchier



Yoshihiro Kawaoka



Hot zone. NIF's laser beams heat the inside of the hohlraum (illustration) to create an x-ray oven.

ENERGY

Laser Fusion Project Alters Goals, Fueling Concern Over Its Strategy

The hopes of fusion researchers worldwide are pinned to the fortunes of the National Ignition Facility (NIF), a \$3.5 billion laser center at Lawrence Livermore National Laboratory in California, which is trying to show that it is possible to create a self-sustaining fusion reaction that could serve as a source of energy. But a change in its milestones, revealed in an internal NIF document *Science* has seen, has highlighted the concerns of some in the field that the path NIF researchers have taken may not be the right one.

Funded by the U.S. Department of Energy (DOE), NIF aims to use a huge laser, the highest energy laser in the world, to crush a tiny fuel capsule to temperatures and pressures higher than in the center of the sun. The goal is to spark a fusion fire in the center of the fuel to start it burning. But the fuel is not considered ignited until high-energy alpha particles produced by the fusion burn heat the surrounding fuel enough to keep the burn going until a significant fraction of the fuel is used.

Until last month, NIF was scheduled to show evidence of significant alpha-particle heating by the end of March, then prove by the end of June that fusion can produce as much energy as the laser provided (known as gain=1), and finally produce an overall energy yield of 5 megajoules (MJ) by the end of September—the end of NIF's initial research program called the National Ignition Campaign (NIC). But a document known as a Baseline Change Proposal, signed by NIF Director Edward Moses on 21 December 2011, would delete this third goal from the NIC schedule and push its two remaining milestones back by 3 months each.

Moses said this week that the changes

were a scheduling, not a physics, issue. NIF has other priorities apart from fusion ignition: It simulates nuclear explosions to help ensure the effectiveness of the nuclear stockpile and is also used for basic science experiments. Moses says there is high demand from these other users. "People wanted more nonignition shots in [early 2012]," he says, so he had to negotiate a new schedule with NIF's funders in the National Nuclear Security Administration.

Moses also downplays the significance of eliminating the 5-MJ milestone. Gain=1 has, since the early planning of NIF in the 1990s, been the working definition of fusion ignition. Once NIF gets the fuel burning, it should just be a matter of finessing the implosion to increase the yield to 5 MJ, Moses says. Others agree that gain=1 is the key target, but some doubt even that milestone will be achieved by the end of the NIC in September. And that would be politically embarrassing for NIF because, as the campaign's name suggests, ignition is one of the key goals of the NIC.

NIF has been controversial from the start. Fusion scientists were only able to propose such a big and expensive machine because of its parallel role in nuclear weapon stockpile stewardship. But some argued that the experimental fusion program was too ambitious and too great a leap from existing techniques (*Science*, 17 April 2009, p. 326).

Since the facility's completion, many have hailed NIF as a technical triumph, but that hasn't stopped the controversy. One bone of contention is the schedule-driven NIC itself, which sticks to a set path in order to get to ignition as quickly as possible (*Science*, 28 October 2011, p. 449). The strategy chosen for the

NIC is a technique known as indirect drive. The 192 laser beams are shot into the ends and onto the inside walls of a small gold cylinder called a hohlraum. The energy they deliver turns the hohlraum into a hot oven filled with x-rays. A plastic sphere the size of a peppercorn containing the deuterium-tritium fuel sits at the hohlraum's center, and the x-ray heating causes the plastic to explode, forcing the fuel inward and compressing it. Getting that implosion just right to cause ignition is hugely complicated and depends on many variables in the beam, hohlraum, and capsule.

Retired physicist Stephen Bodner, former head of laser fusion at the Naval Research Lab, is one of those concerned about the NIC. Based on presentations by NIF researchers at plasma physics meetings, Bodner concludes that indirect drive "is almost certain to fail." NIF researchers "have not really faced up to the problems they have," he says.

One of the strengths of indirect drive is that converting the laser light to x-rays in the hohlraum smooths out imperfections in the laser beams, although this conversion does waste much of the beams' energy. That was expected, but critics point out that other processes in the hohlraum seem to be diverting more energy from the job of imploding the capsule. "Energy is going in places they don't want or in forms they don't want it," says a fusion researcher who asked not to be identified.

Even former DOE Under Secretary for Science Steven Koonin had doubts about NIF's progress. In a memo dated 8 November 2011, following his fourth review of the NIC, Koonin noted: "Surprises encountered on the path to ignition make it impossible to predict confidently the rate of progress on those issues of greatest concern to the NIC and so ignition by the end of [fiscal year 2012] is not assured."

Bodner and researchers in other laser fusion labs favor the alternative approach of direct drive: shining NIF's laser beams straight onto the fuel capsule. Although this demands higher beam quality, more beam energy goes into compressing the fuel.

The NIF team is making good progress, according to Moses. "It's a very hard problem," he says. "But we have the capability to do good experiments on the path to ignition." The team has carried out 15 realistic shots with damped-down fusion fuel, improving the quality of the implosion by a factor of 100, Moses notes. "There's another factor of 10 to go. If it's there, we'll get it."

—DANIEL CLERY

OCEANOGRAPHY

China Makes Waves With Ambitious Ocean Research Plan

SHANGHAI—Last July, when the *Jiaolong* submersible plunged 5000 meters below the Pacific Ocean waves, China joined an elite set of nations capable of crewed exploration of the deep sea. *Jiaolong*'s program this coming summer will be even more eye-catching. First, the sub will attempt a world record-breaking dive 7000 meters below sea level; then it will carry out its first research mission in a region of high scientific and political significance: the South China Sea.

The target is enticing. Its sediments hold clues to the evolution of the East Asia monsoon and how tropical conditions over the

And China is leading an Intergovernmental Oceanographic Commission (IOC) project on the dynamics of as much as 570 million tons of sediments that rivers discharge into the South China Sea each year.

But politics cast a shadow over these efforts. China claims sovereignty over a wide swath of the South China Sea, which has sparked territorial disputes with six neighbors. At stake are rich fishing grounds and seabed resources, including gas hydrate reserves. Last November, U.S. Secretary of State Hillary Clinton irritated China when she offered U.S. help in mediating disputes; China reiterated its stance that it would negotiate only bilaterally. Meanwhile, a few Chinese scientists have raised hackles by including

in publications maps depicting China's territorial claims—reportedly at the government's behest.

“Southeast Asian nations are carefully monitoring the expanding geopolitical influence of China in this region. One is aware that scientific data could be crucial in supporting territorial claims,”

says Edlic Sathiamurthy, a hydrologist at University Malaysia Terengganu who is involved in the IOC sediment study.

Long-simmering tensions complicate research. “There are serious difficulties as a result of territorial claims,” Sathiamurthy says. Sampling in Malaysia's Exclusive Economic Zone, he says, requires approval from the National Security Council. When working with foreign colleagues, Sathiamurthy says, “we are expected to make sure national interests and security are safeguarded.”

Scientists involved in South China Sea Deep, which is funded by the National Natural Science Foundation of China, say there is no hidden agenda and pledge transparency. “Data will be shared freely between scientists from different countries,” says Wang Pinxian, a marine geologist here at Tongji University.

South China Sea Deep is the culmination of 2 decades of patient efforts to persuade China's leaders to pay attention to ocean research. “Deep-sea studies were overlooked for many years,” says Wang, China's preeminent ocean scientist. A breakthrough

came in 1999, when Wang co-led an Ocean Drilling Program research cruise in the South China Sea. That and subsequent seabed coring expeditions have yielded a trove of paleoclimate data, putting the South China Sea on a par with the Arabian Sea as a place to decipher ancient monsoon patterns, says Jian Zhimin, director of Tongji's State Key Laboratory of Marine Geology.

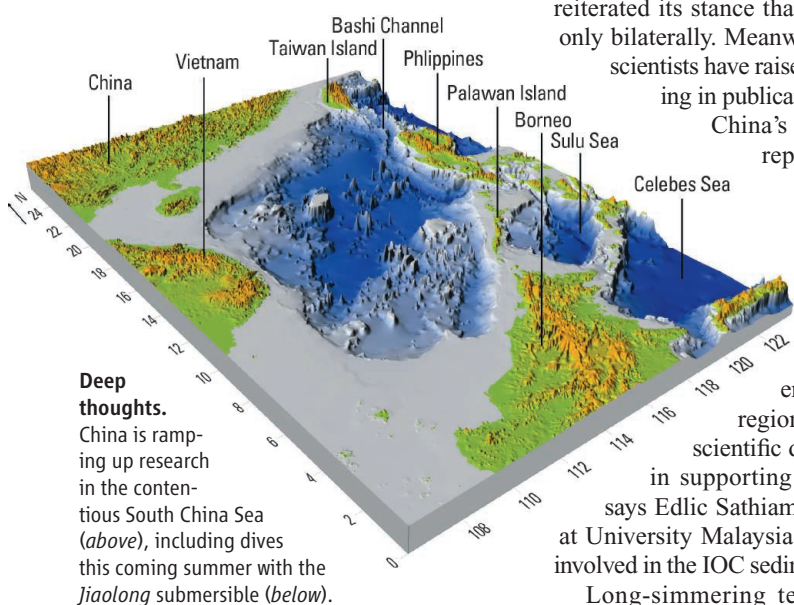
Then a few years ago, the central government grew keenly interested in ocean research. “Because of the economy,” Wang explains. “If the seaways are cut off, we can't import oil. China would be dead.” Another incentive was China's discovery in 2007 of gas hydrates in the South China Sea. This summer's planned 10-day *Jiaolong* program will explore gas hydrate outcrops at a depth of around 1000 meters in the South China Sea.

Jiaolong's dives will get South China Sea Deep off to a flying start. Over the next decades, scientists will drill into the seabed and use deep-tow measurements to estimate the oceanic crust's age, probe a possible mantle plume off Hainan Island, and explore the origins of volcanic chains. They will also sample methane seeps and characterize microbes in the water and sediments. “The project is expected to reveal the life history of the South China Sea,” Wang says.

Researchers here at Tongji, a powerhouse in ocean research, note that South China Sea is not their sole target. Last month, they broke ground on a marine science center at the tip of the Yangtze River delta. It will be a staging ground for an \$80 million sea-floor observatory just off shore, in the East China Sea. And they are eager to collaborate broadly with scientists from Southeast Asian nations, says Tongji marine geologist Liu Zhifei, who leads the IOC sediments project. Last November, for example, Tongji and the Hanoi University of Mining and Geology agreed to cooperate on education and research in marine geology and geophysics. “Our goal is to develop marine geology in Asia, not just China,” Wang says.

Southeast Asian researchers welcome such overtures and say they see eye to eye with their Chinese colleagues. Research in the South China Sea “has a potential to ease political tensions if every scientist is transparent and does not serve some greater geopolitical agenda,” Sathiamurthy says. “Then again,” he says, “it would naïve not to be careful.”

—RICHARD STONE



Deep thoughts. China is ramping up research in the contentious South China Sea (above), including dives this coming summer with the *Jiaolong* submersible (below).



eons have influenced global climate patterns. To tackle these questions, China has just embarked on South China Sea Deep, an 8-year, \$24 million project feted last month at the American Geophysical Union's fall meeting in San Francisco. Complementing *Jiaolong*'s dives will be research cruises in the area this spring with France and Germany.

U.S. SCIENCE

Research Remains a Favored Child in Budget Decisions

Another year, another decent budget.

One year after Republicans won control of the House of Representatives by promising to slash federal spending, the budgets of most research agencies have been left largely intact (*Science*, 23 December 2011, p. 1613). Even more striking is a comparison of the new 2012 budgets with those from 2010, when Democrats last controlled both houses of Congress and the presidency. Science has not only held its own but actually done better than most of the rest of the federal budget.

To be sure, 2012 was not the banner year the Obama Administration had envisioned last February, when it asked Congress to give double-digit increases to several agencies as part of an ongoing effort to promote the physical sciences and clean energy (*Science*, 18 February 2011, p. 832). And while few lobbyists expected a high-growth plan to hold up in these tight fiscal times, the budget disaster some warned of in 2012 did not come to pass either. Instead, legislators on both sides of the aisle agreed to respect most of the Administration's priorities and protect academic research from serious disruption even as so-called discretionary spending—the portion of the budget Congress can directly control—fell by about \$50 billion (roughly 5%) over those 2 years.

That balanced philosophy applies nearly across the board (see graph). The new budget for the \$7 billion National Science Foundation (NSF), for example, is 2.3% larger than in 2010 (without factoring in inflation)—and 8.5% bigger than in a plan unveiled last January by House Republicans. The Department of Energy's (DOE's) Office of Science has the same amount to spend this year, roughly \$4.9 billion, as in 2010—but that's \$900 million above last January's proposal. The behemoth of U.S. basic research, the National Institutes of Health, does suffer a 1% cut from 2010 levels. But its decline is much less than the 4% drop sought by the House GOP leadership. The current \$5 billion science exploration budget at NASA is actually 13% bigger than it was in 2010. And the Department of Defense, the fifth largest supporter of basic research in the federal government, has seen its research account, known as

6.1, jump by 11.2% in 2 years, in line with a goal set by former Defense Secretary Robert Gates to allocate 3% of the military's overall budget to basic research.

Despite their perennial hand-wringing, most science lobbyists feel that Democrats and Republicans are equally well-disposed to their arguments. "In a year when money is

political risk. "Nobody gets criticized for being a supporter of science," he notes.

A third factor is the decentralization of science funding across the federal government. Funding for science is taken up piecemeal by Congress each year as it determines the budgets for individual agencies, a dozen of which support more than \$100 million a year in research. While in the past some have advocated a Department of Science as a more efficient mechanism for managing federal dollars, the current arrangement serves two important purposes. One, it builds a bigger political constituency for research by making science part of the portfolio of many federal agencies and congressional committees. Two, it creates steep obstacles to any attempt to curb spending across the government on any particular research topic.

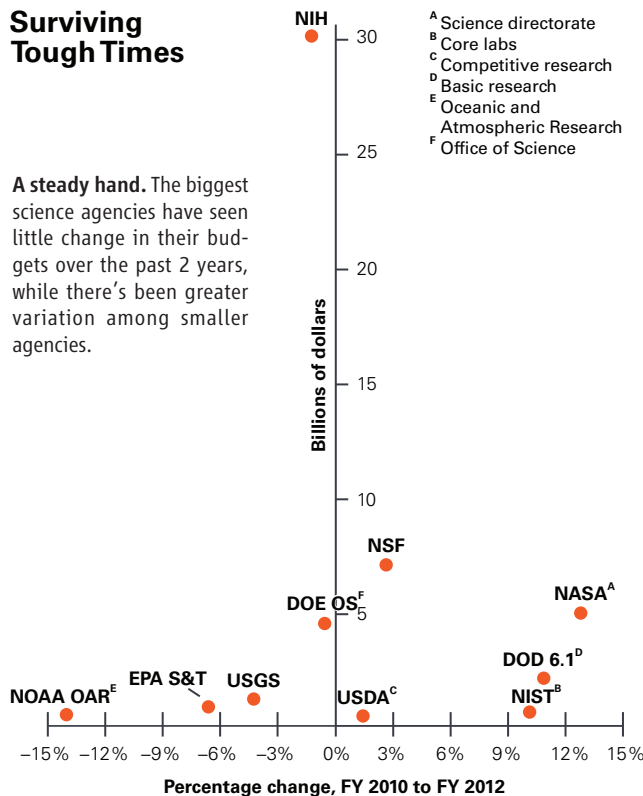
This year's assault by House Republicans on climate science, for example, was blunted by the fact that research on the topic is supported by several federal agencies. Opponents had to settle for knocking out the Administration's plans for a National Climate Service within the National Oceanic and Atmospheric Administration, a tiny piece of the government's overall \$2-billion-a-year climate change research program.

Sometimes opposition to one type of research even redounds to the advantage of other research programs. That was part of the dynamic this year in the House, where Republicans fought the Administration's plans to increase applied energy research. "We believe the Office of Science should be the top funding priority among DOE R&D programs and be protected from cuts by the Joint [Super] Committee," the House Committee on Science, Space, and Technology explained in an October letter to the so-called Super Committee, arguing that the office was a higher priority than the fledgling Advanced Research Projects Agency-Energy (ARPA-E), a favorite of Energy Secretary Steven Chu. In the end, however, ARPA-E more than held its own, receiving \$275 million—half of the Administration's request but 50% more than its current budget.

The Super Committee was created this

Surviving Tough Times

A steady hand. The biggest science agencies have seen little change in their budgets over the past 2 years, while there's been greater variation among smaller agencies.



summer by Congress to reach a bipartisan agreement on a 10-year deficit-reduction plan, and its abject failure has triggered automatic cuts that are supposed to go into effect starting in January 2013. The process laid out in the Budget Control Act, enacted in August, calls for “sequestration” or spending cuts of roughly \$1 trillion over 9 years, divided between domestic and military discretionary programs. That would remove \$35 billion a year from the accounts that fund

all nondefense programs, including all civilian science activities.

That number is the basis for warnings from science lobbyists that agency science budgets could be slashed by 7% to 8% in 2013 and by a similar amount in subsequent years. On the other hand, the sequestration’s delayed start gives Congress and the White House a full year to come up with another way to meet the Budget Act targets for reducing the deficit. If Congress finds an alternative to the automatic

cuts, then all bets are off on what science agencies will receive in 2013 and beyond.

Of course, 2012 is also an election year. And the betting is that partisan politics will squelch any attempt by Congress and the White House to reach a long-term budget agreement, at least until after the November election. But no matter which party controls the levers of power in 2013, recent history suggests that science will survive.

—JEFFREY MERVIS



Big plans. New York Mayor Michael Bloomberg (left) congratulates the presidents of Cornell and Technion on their winning proposal for an applied science campus in the city.

U.S. GRADUATE EDUCATION

Cornell's Plans for the Big Apple Rely on Quality, Cash, and Dreams

Now comes the hard part. After besting some of the world's top universities in a months-long competition and winning access to some of the choicest real estate on Earth, Cornell University and Technion-Israel Institute of Technology must actually build the applied research university that, they hope, will transform New York City into Silicon Valley East.

In December 2010, New York Mayor Michael Bloomberg offered land and \$100 million in incentives to institutions willing to build a new graduate applied science and engineering school in the city. Seventeen groups of elite research universities—from the United States, India, South Korea, Canada, and Europe—submitted proposals, and Stanford University, with its roots in Silicon Valley, was widely touted as the favorite.

On 19 December, only 3 days after Stanford unexpectedly pulled out of the competition, Bloomberg chose Cornell and Technion's bid to build a 4.5-hectare campus on Roosevelt Island, between Manhattan and Queens. It certainly didn't hurt that Cornell

pledged to invest \$2.1 billion over 30 years in the project, nor that its president, David Skorton, demonstrated the university's fundraising prowess by announcing a \$350 million gift from billionaire Charles Feeney only a few hours after Stanford bowed out.

The new campus, dubbed NYCTech, could support up to 280 faculty members and 2500 students by the 2040s, say Cornell officials. These students and researchers would focus on science, mathematics, and computer science with commercial applications that the city hopes will generate \$23 billion in economic activity over 30 years, including \$1.4 billion in taxes. Their presence would increase the number of graduate engineering students in New York City by 70%, according to the mayor's office.

Cornell has proposed creating a series of interdisciplinary hubs, says Daniel Huttenlocher, dean of Cornell's school of computing and information science, who helped craft the school's bid. The hubs will likely change each decade, he says. The ini-

tial trio will consist of “connective media,” focused on providing new ways for people to interact socially; “healthier living,” focused on providing technologies for day-to-day and preventative medicine; and the “built environment,” focused on making our daily physical environment more energy-friendly or comfortable.

Huttenlocher says the hubs will have their roots in traditional academic departments, especially applied mathematics, computer science, operations research, information science, and electrical and computer engineering. Those choices play to Cornell's strengths, he says. The latest National Research Council rankings of U.S. graduate programs, drawn from data collected in 2006, list Cornell among the top five in applied math, the top 10 in computer science and in operations research, and the top 30 in electrical engineering. These rankings compare quite favorably with the city's current lineup of research universities (see graphic).

Opening a New York City satellite campus will also help Cornell attract students and faculty, says Steven Pedigo, director of research at Creative Class, a think tank that studies urban life. Cornell's hometown of Ithaca “is sort of isolated,” he says, but New York City has amenities such as hip restaurants and art institutions that creative young people crave. Plus, Cornell officials say the university's medical school in Manhattan and the 50,000

regional alumni give it strong ties to the city.

At the same time, Cornell will need to grow significantly to meet its goals. The five departments that will form the core of the new campus contain about 200 faculty members and enroll roughly 750 graduate students. So a fully operating NYC campus would more than double the current faculty and more than triple graduate enrollment in those fields. Huttenlocher says that the home programs in Ithaca will likely grow as well, requiring even more hires.

Reaching those targets won't necessarily transform New York City's economy, however, says Gina Neff, an assistant professor in the department of communication at the University of Washington, Seattle, and author of *Venture Labor*, an upcoming book about New York's tech boom in the 1990s. Neff says that NYCTech must partner with established New York City industries to exert the economic impact that Bloomberg envisions. Cornell's three hubs, as described by Huttenlocher, shrewdly target the city's strong media, health care, and design industries. But Neff notes that "it takes so many factors to get to an innovative region. If building a campus alone were the key, many cities would have already gotten there."

Take Pittsburgh and Urbana-Champaign. Neff says Carnegie Mellon University and the University of Illinois provide those cities with "amazing engineering centers," but no one would call them tech capitals. "I suspect Cornell's leadership has a different agenda in wanting to advance Cornell University and being able to plant a larger flag in New York," she says. But increased name recognition, prominent faculty members, and supportive alumni won't necessarily translate into thousands of new jobs for the region, she warns.

New York City's high rents and red tape make it a difficult place to launch new businesses, she adds. Entrepreneurs have complained to her that even arranging for utilities can be a struggle.

Huttenlocher admits that "it's not a given

that great students will stay nearby" and work in New York. But the requirement that students work with industry mentors during their graduate training should improve the chances that they will remain in the region after graduation, he predicts. NYCTech will also provide up to \$150 million in seed money to local start-ups. As a comparison, roughly 26% of Massachusetts Institute of Technology engineering graduates chose Massachusetts as the site of their start-up company, according to a recent study, and Huttenlocher says NYCTech is aiming to better that mark. The mayor's office hopes NYCTech will produce 600 start-ups over 3 decades, generating 30,000 permanent jobs.

Technion's role in the campus appears to be minor compared with Cornell's. It will put up very little of the cost of construction and operations, and as of now plans

to offer only one joint master's degree, with Cornell, in applied science. Technion's biggest contribution, Huttenlocher says, will be to bring a more global perspective to the campus and also to make it more likely that research done there eventually translates into jobs and economic activity. In the past few decades, Israel has transformed itself from an agrarian economy to a rich high-tech economy, and Huttenlocher says Technion provided much of the skilled workforce needed to accomplish that transformation.

Despite its small size, Israel ranks fourth—behind only the world's top two economies (the United States and China) and Canada—in the number of companies listed on the NASDAQ stock exchange. Half of those NASDAQ companies are run by Technion graduates, says the school, and companies headed by Technion alumni employ about 85% of the high-tech employees in Israel. The school's technology transfer office also files for roughly 300 patents per year.

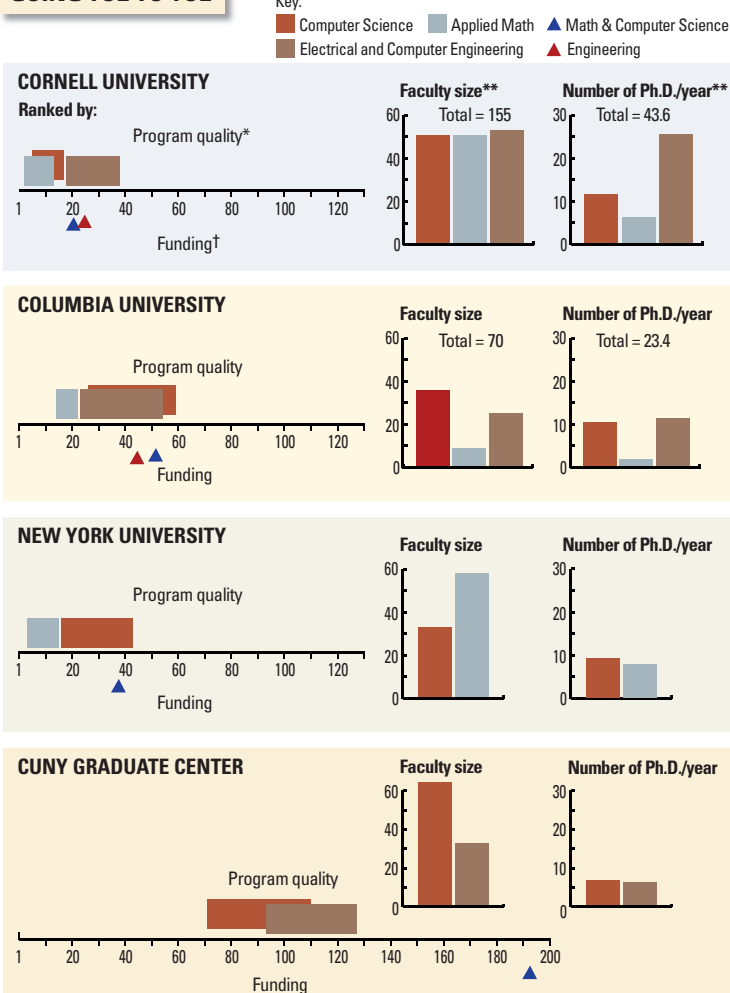
However, some question how much credit Technion should receive for fueling the country's tech boom, which drew from many sources. Daniel Isenberg developed

the first course on technology-based entrepreneurship at Technion in 1987 and is now a professor of management practice at Babson Global, a subsidiary of Babson College in Wellesley, Massachusetts. "It wasn't like Technion set up the companies or did anything to foster them," he says.

One sure bet is that the campus will transform sleepy Roosevelt Island, and quickly: NYCTech plans to open its first building there in 5 years. (Cornell has also promised to start offering classes next fall, in temporary quarters.) But it remains to be seen whether NYCTech can truly transform the city's economic identity. Bloomberg's attempt to create a tech boom with one project is "a bit chauvinistic," Isenberg says. "It's the mayor's dream," he adds, "but it's not clear if he can have his dream."

—SAM KEAN

GOING TOE TO TOE



*R-rank in 2010 NRC doctoral programs report **2010 NRC report †2009 research expenditures, NSF

Coming to NYC. The programs that Cornell University expects to jump-start its new graduate campus on Roosevelt Island compare favorably to those at three existing universities in the city on overall quality, research funding, size of faculty, and annual production of Ph.D.s.

Unraveling the Obesity-Cancer Connection

A growing body of research shows that insulin and a related hormone play a key role in fueling tumors. They also may be a link between obesity, diabetes, and cancer

GROWING BREAST CANCER CELLS IN THE lab has been a revelation to Vuk Stambolic. The protocol he follows is decades old and widely used, but there's a puzzle at its core. The recipe calls for a large dose of glucose, a growth factor called EGF, and insulin. Add these to tissue culture, and tumor cells will be fruitful and multiply. A curious thing happens if you try to wean the tumor cells off insulin, however: They "drop off and they die," says Stambolic, a cancer researcher at the University of Toronto in Canada. "They're addicted to [insulin]."

What makes this so "bizarre," Stambolic says, is that this behavior is totally unlike that of the healthy breast cells from which

these tumor cells are derived. Normal cells are not sensitive to insulin—or at least not nearly to the same degree. They don't have insulin receptors, and they lack key elements of the insulin signaling pathway necessary to make insulin outside the cell immediately relevant to what goes on inside. Indeed, normal cells thrive without insulin. By contrast, the tumor cells in culture can't live without it.

This observation, although not original, is one of the insights that drew Stambolic to investigate the tumor-promoting effects of insulin. It has led him to spend the past decade studying a signaling pathway that is activated by insulin in healthy muscle, fat,

and liver cells. Named for one of its key components—the PI3 kinase pathway—it also happens to be among the most frequently mutated pathways in human cancers.

Insulin, a hormone produced in the pancreas, is more commonly known for its role in diabetes. But its reputation may be changing. Insulin and a related hormone known as insulin-like growth fac-

High burn. A PET scan lights up the brain where cancer cells are consuming glucose at a rapid rate.

tor (IGF) are now at the center of a growing wave of research around the world aimed at elucidating what many scientists consider to be their critical role in fueling a wide range of cancers. Elevated levels of insulin and IGF are also the leading candidates to explain a

Online

sciencemag.org

S Podcast interview with author Gary Taubes.

significant correlation in epidemiology that has gained attention over the past 30 years: Obese and diabetic individuals have a far higher risk than lean

healthy people of getting cancer, and when they do get it, their risk of dying from it is greater. And now that obesity and diabetes rates are skyrocketing, the need to understand this link has become far more urgent.

The correlation between obesity and cancer can be found in the medical literature going back for several decades. But it wasn't until 2004 that two cancer epidemiologists put it all together, says Robert Weinberg, a cancer researcher at the Massachusetts Institute of Technology (MIT) in Cambridge. An article that year in *Nature Reviews Cancer* by Rudolf Kaaks, then of the International Agency for Research on Cancer, and the late Eugenia

Peculiar dependency. Vuk Stambolic says that breast tumor cells seem "addicted" to insulin.



Continued on page 30

Continued from page 28

Calle of the American Cancer Society “laid down a challenge to the rest of us ... to determine why obesity is such an important determinant of cancer risk,” Weinberg says.

The message of this research is straightforward, Kaaks says: Excess body fat seems to account for between one-quarter and one-half of the occurrence of many frequent cancer types—breast, colorectal, endometrial, renal cell, and adenocarcinoma in the esophagus, in particular. Kaaks adds, “The list is growing.”

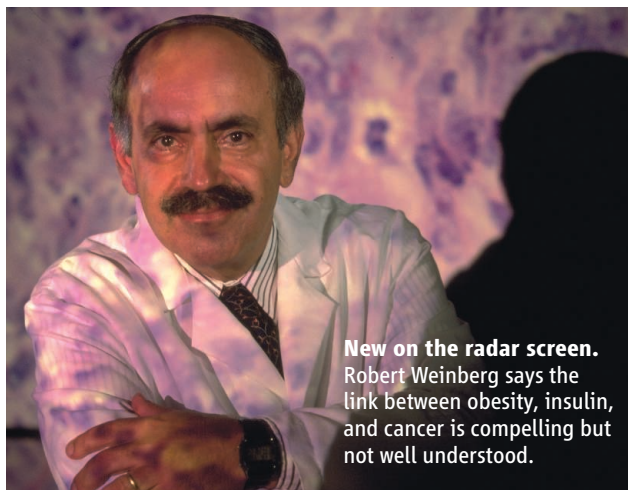
“The magnitude of the effect is huge,” in large part because obesity and diabetes are now so common, says Michael Pollak, an oncologist at McGill University in Montreal, Canada. It seems that cancer “loves the metabolic environment of the obese person,” Pollak says. Epidemiologic studies have also found that not only is type 2 diabetes associated with increased cancer incidence and mortality but so are circulating levels of insulin and IGF.

Recent drug studies have sharpened the picture: Type 2 diabetics who get insulin therapy or drugs to stimulate insulin secretion have a significantly higher incidence of cancer than those who get metformin, a drug that works to lower insulin levels (see sidebar on metformin, p. 29). There’s a large and growing body of evidence implicating insulin and IGF in cancer, Pollak says, “and it’s causing a lot of people to stay up at night thinking about it.”

Parallel worlds

Researchers have recently upped their interest in the idea that insulin and IGF drive cancer in part because other hypotheses of cancer causation have failed to pan out. W. Robert Bruce, for instance, a cancer researcher at the University of Toronto, embarked in the late 1970s on what he described as a lengthy and fruitless search for mutagens in the diet and environment that might be responsible for colon cancer. “About a ton of feces later,” he says, he had found nothing.

Now many cancer researchers, including Bruce, have come to believe that, whatever the carcinogenic substances or factors are, they mostly work not by directly damaging DNA but by promoting tumor development through a change in



New on the radar screen. Robert Weinberg says the link between obesity, insulin, and cancer is compelling but not well understood.

the hormonal environment around incipient tumor cells, increasing, for instance, insulin and IGF levels in the circulation. “There is a change in the endocrine and growth factor environment of cells,” Kaaks says, “that pushes cells to proliferate further and grow more easily” and to evade built-in programs that cause normal cells to die.

To learn about the research on insulin, IGF, and cancer, Bruce says he had to read the diabetes literature—and what he found was a parallel universe. “It was a complete edifice of research in and of itself, not linked by any papers with the edifice of research in the cancer field—two big towers.”

A few bridges have been built between these two parallel worlds, however. One connects cancer to diet and obesity. It was not obesity’s harmful effects that first drew cancer researchers’ attention but the flip side: the observation that tumor growth in animals is inhibited if not prevented entirely if the animals are semistarved. Peyton Rous, who would later win the Nobel Prize for his

discovery of tumor-causing viruses, was the first to make the observation. It was confirmed in 1942 by Albert Tannenbaum, a Chicago pathologist, who demonstrated that feeding rats a diet just sufficient to keep them alive markedly increased their life span, in part by inhibiting tumors.

Tannenbaum suggested that a likely mechanism was a phenomenon known as the Warburg effect, in which cancers adopt an inefficient type of metabolism commonly used by bacteria, known as aerobic glycolysis (see sidebar, p. 31). It goes along with a significant increase in the use of glucose for fuel by the cancer cells. In semistarved, growth-stunted animals, Tannenbaum proposed, the tumors could not obtain the huge amounts of blood sugar they need to fuel mitosis, division of the nucleus, and continue proliferating.

In the decades since, researchers have debated whether the amount of blood sugar available to the tumor could be a driving or limiting factor in tumor development. But positron emission tomography scans of patients given fluorodeoxyglucose, a traceable analog of glucose, show that tumors continue to burn high amounts of glucose even if the blood glucose levels in the patients themselves are relatively low. “There’s always plenty of glucose around,” says Chi Dang, a cancer researcher at Johns Hopkins University (JHU) in Baltimore, Maryland, “so it’s got to be something else” fueling the tumors.

Cancer accelerant

That insulin and IGF may be the relevant “something else” that fuels cancer is a relatively new idea. But the evidence, as Bruce points out, has been accumulating for decades. In the mid-1960s, researchers demonstrated that insulin acts as a promoter of growth and proliferation in both healthy and malignant tissues. By the late 1970s, C. Kent Osborne, then at the National Cancer Institute, and his colleagues reported that a line of particularly aggressive breast cancer cells were “exquisitely sensitive to insulin” and that breast cancer cells express insulin receptors, even though the cells from which the tumors derive do not.

“You find the highest level of insulin receptors in liver, muscle, and fat tissue naturally,” says Lewis Cantley, director of the Beth Israel Deaconess Medical Center at Harvard Medical School in Boston. Cantley originally trained as a biophysical chemist and found himself working on the link between obesity, diabetes, and cancer when he started studying

Bad signals. Lewis Cantley suspects that a dysfunctional PI3K pathway may be behind many cancers.



CREDITS (TOP TO BOTTOM): © BROOKS KRAFT/SYGMA/CORBIS; BRUCE WAHL

Warburg effect. Healthy tissues (*left*) get energy through the efficient process known as oxidative phosphorylation. Tumors (*right*) use aerobic glycolysis, the so-called Warburg effect, which is inefficient but seems to enable proliferation.

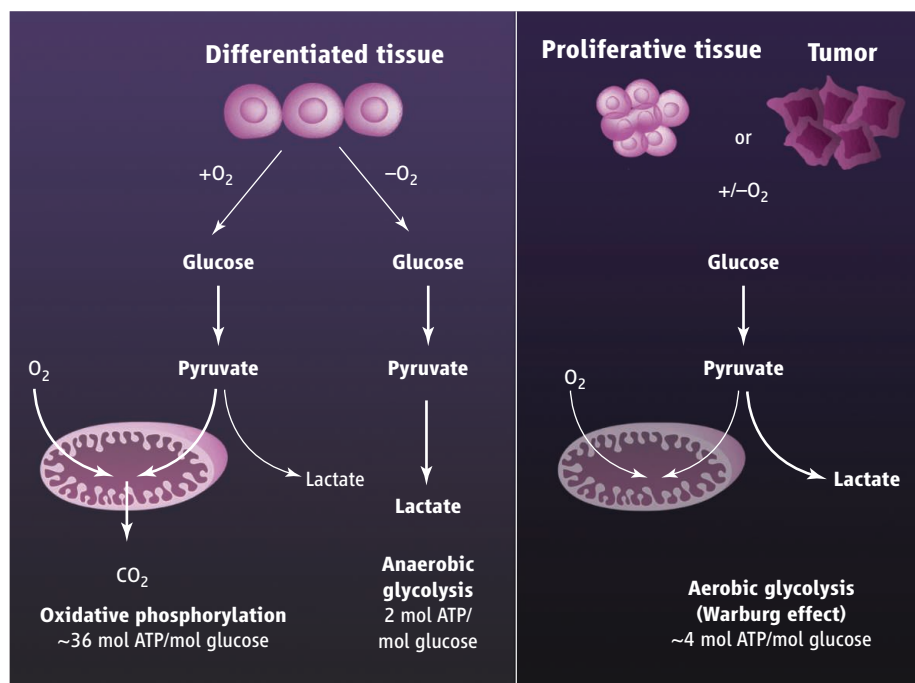
how hormones and growth factors regulate cell metabolism. Low levels of insulin receptors, he says, can be found in half a dozen other healthy tissues as well, but that's it. So the abundant presence of insulin receptors in prostate cancer, colorectal cancer, and breast cancer cells, among other cancers, is significant, Cantley says: "They must be there for a reason; they must be helping to grow the tumor. And one thing they're doing is giving cancer cells the ability to take up glucose at a higher rate."

The second suspect in this scenario, the hormone IGF, was discovered in the late 1950s; its designation "insulin-like" wasn't made for another 20 years after that. IGF's structure is similar to that of insulin, and its effects can mimic those of insulin. But its secretion is stimulated by growth hormone, says Derek LeRoith, a diabetologist who now runs the Metabolism Institute at the Mount Sinai Medical Center in New York City.

In the early 1980s, researchers discovered that tumor cells typically have two to three times as many IGF receptors as healthy cells, making them much more responsive to the IGF in their immediate environment. In rodents, functioning IGF receptors appear to be a virtual necessity for cancer growth, according to Renato Baserga of Thomas Jefferson University in Philadelphia, Pennsylvania, who "stumbled" upon the discovery in the late 1980s. Shutting down the IGF receptor in mice leads to what Baserga calls "strong inhibition, if not total suppression of [tumor] growth"; it is particularly lethal to tumors that have already metastasized from a primary site elsewhere in the body.

LeRoith has genetically engineered mice so that their livers do not secrete IGF, resulting in one-quarter of the IGF concentration in their circulation compared with normal mice. When colon or mammary tumors are transplanted into these mice, according to LeRoith, both tumor growth and metastasis are significantly slower than when identical tumors are implanted in normal mice with normal IGF levels. When IGF is injected into these genetically engineered mice, tumor growth and metastasis accelerate.

The consensus among those researchers studying the role of insulin and IGF in cancer is that these hormones supply both the fuel necessary for tumors to divide and multiply and the signals to continue doing it.



Ravenous for Glucose

The focus on obesity, cancer, and hormones has kindled a wide interest in the metabolism of cancer cells and particularly in work done in the 1920s by the German biochemist and later Nobel laureate Otto Warburg. Warburg observed that tumor cells can survive without oxygen and generate energy by a relatively inefficient process known as aerobic glycolysis. This conversion of cancer cell metabolism to aerobic glycolysis has been known as the Warburg effect ever since. It is akin to how bacteria generate energy in the absence of oxygen, although cancer cells do it even when oxygen is present (hence "aerobic"). Rather than converting glucose to pyruvate and burning that with oxygen in the cells' mitochondria, the pyruvate is converted to lactate in the cells' cytoplasm outside the mitochondria, and no oxygen is used. The process yields only one-ninth the energy, four ATP molecules instead of 36, from each molecule of glucose.

One result is that cancer cells have to burn enormous amounts of glucose to thrive and multiply. This abnormally high glucose consumption is what's detected by the imaging technology known as FDG PET when it's used to identify where tumors might have spread in the body. Warburg hypothesized that the high-glucose metabolism is what drives cancer. But there has always been, and still is, significant controversy about why cancer cells use it: What's in it for them, if it's such an inefficient means of supplying energy? And how can we tell whether it is a byproduct of the cancer or a cause? Most researchers studying the Warburg effect now believe that the signaling pathways driving it are the insulin and insulin-like growth factor pathways. The question they're still hoping to answer is which comes first: the metabolism change or the cancer?

—G.T.

A third conspirator

An additional player has been identified as a key member of this particular network that influences metabolism, growth, and cancer: the enzyme PI3 kinase, discovered by Cantley and his colleagues in the mid-1980s. PI3K lies in the insulin signaling pathway and is activated by both insulin and IGF. Through its effect on other molecules, PI3K effectively regulates a cell's sensitivity to insulin. When PI3K is activated, insulin is more effective at stimulating the transport of glucose into cells.

PI3K also turns out to play a major role in cancer—a discovery that came in a series of

steps in the late 1990s. The finding that "put PI3K on everybody's radar screen as something important in human cancer," Cantley says, was the realization that it is linked with a tumor suppressor gene called *PTEN*. Identified in 1997, *PTEN* is "the most frequently deleted gene in a whole host of advanced human cancers," Cantley says.

When researchers set out to elucidate what exactly the intact *PTEN* was doing to suppress tumors, they learned that it counteracts the work of PI3K. It removes a phosphorus atom from the fat molecule that PI3K makes, an effect equivalent to decreasing the influ-

ence of PI3K itself. And it turns out, as Victor Velculescu, a geneticist at JHU, has demonstrated, PI3K itself is commonly mutated—in a way that bypasses normal mechanisms for turning it off—in colon cancer and a host of other cancers as well, including breast, lung, brain, and ovarian cancers.

The point, Cantley says, is that researchers have identified two general ways that work to step up activation of the PI3K pathway: by mutations such as those that alter *PTEN* or by abnormally elevated levels of insulin and IGF in the circulation. Most obese individuals have elevated insulin and IGF levels, as do type 2 diabetics. When PI3K signaling is increased, cells take up more glucose and may convert to a high-glucose metabolism—the aerobic glycolysis described by Warburg. It may be an inefficient means of generating energy, Cantley says, but it doesn't matter to the cell because the insulin makes sure it has considerable glucose to burn.

So what's in it for the cancer cells? The answer, according to Cantley, appears to be that the carbon backbones of the glucose molecules are shunted aside during aerobic glycolysis rather than burned for fuel, and these carbon backbones can then be used to make new fatty acids.

Normal fat cells do the same thing when they burn glucose: They preserve the carbon backbone for storing fatty acids as triglycerides, Cantley says. In cancer cells, the fatty acids are used to build new membranes for daughter cells. The glucose is also used to make new DNA and protein for the cells. So the cancer cells are effectively trading off an inefficient means of producing energy for a means of obtaining the resources necessary to create new cancer cells. It's a tradeoff they can easily afford because there's so much glucose now pouring in. "Remember, cancer cells have to duplicate themselves," JHU's Dang says. "So this way you see the interplay between energy production and at the same time providing the skeletons, the building blocks for the cancer cells."

These researchers now suggest that it may make sense to divide tumors into two types, like diabetes: insulin-dependent and insulin-nondependent. If there are no mutations enhancing the activity of the PI3K pathway, Pollak says, then the cancer process will be dependent on the insulin and IGF in the circulation. "But if PI3K is mutated," he says,

"that cell is going to be highly proliferative, highly aggressive, and it couldn't give a damn about the insulin environment."

Recent evidence of the power of this signaling pathway comes from work by David Sabatini of MIT's Whitehead Institute and Nada Kalaany, who's now at Children's Hospital Boston. In 2009, they showed that PI3K appears to determine whether a tumor responds to calorie restriction. When they induced different types of human cancers in mice and then put the mice on semistarvation diets, some of the tumors shrank in response and some didn't. Tumors grew less in the mice with low PI3K pathway activity, more in those with high activity.



Full circle. By tweaking insulin signaling, Craig Thompson and others have induced high-glucose metabolism in mice.

For cancers with one of the mutations that activates the PI3K pathway, Sabatini says, calorie restriction has little to no effect because the insulin signaling is turned on anyway. These cancers are resistant to changes in insulin levels. "In obesity," Sabatini says, "there are many things going on, but one of them is hyperinsulinemia [high circulating levels of insulin], and that is going to be an important driver of tumor genesis in animals or people. It's like mimicking the hyperactivation of PI3K. Instead of doing it by mutation, you do it by having tons of insulin around."

The picture that's emerging now, Dang says—one that's "clearly simplified and needs to be tweaked"—is that many common cancer genes when activated may increase the uptake of glucose and convert the cell to the Warburg-type of metabolism.

Cause or consequence?

This still leaves open the question of what comes first: the Warburg effect or the mutations that drive a cell to adopt it. Craig

Thompson, now president of Memorial Sloan-Kettering Cancer Center in New York City, has been working on this problem for a decade. He believes, as does Cantley, that the likely first step in the progression to cancer is the increase in insulin signaling, which then induces the Warburg effect. Genetic defects follow. Thompson and his colleagues have shown that they can induce the Warburg effect in the cells of healthy mice, or in cells associated with cancer, just by activating PI3K and increasing insulin signaling. "If you put in components of the insulin pathway into these cells," Thompson says, "you get the Warburg effect."

Once this happens and cells have increased their glucose metabolism 10- to 20-fold, Thompson says, one result is a significant increase in the generation of reactive oxygen species—free radicals—that can induce mutations in the genome. Cantley describes it as a vicious cycle. "The faster you do glucose metabolism," he says, "the more likely you are to get free radicals that can damage DNA. ... If the mutations happen to be in *PTEN* or PI3K, that could make the whole system rev up even further, making more free radicals, causing more DNA damage. So you're getting this feed-forward acceleration of tumor growth."

This hypothesis still has plenty of critics—MIT's Weinberg being the most prominent. Insulin and IGF may be the "most attractive mechanisms" to explain the obesity-cancer link, Weinberg says. But he argues that their primary role is not to turn on the Warburg effect or promote proliferation but to suppress cell-suicide mechanisms. "One of the mechanisms," he says, "by which the body protects itself from cancer is by inducing incipient cancer cells to kill themselves by a variety of mechanisms. One of those mechanisms is apoptosis, and insulin and IGF activate an enzyme that in turn emits a series of antiapoptotic signals. A minimal amount of IGF is required just to protect normal cells from killing themselves. They're always poised on the brink."

Still, as Weinberg says, the role of insulin and IGF in cancer only "recently came on the radar screen" of most cancer researchers. "The epidemiology connecting obesity with cancer is very compelling," he says. But "our understanding of the mechanism is still pretty soft."

—GARY TAUBES

CREDIT: PHOTO COURTESY OF MEMORIAL SLOAN-KETTERING CANCER CENTER



Cancer Prevention With a Diabetes Pill?

In 2005, Andrew Morris and his colleagues at the University of Dundee in the United Kingdom were following up on therapy for type 2 diabetes patients when they reported results that have since set the world of cancer research abuzz. They found that use of an insulin-lowering drug known as metformin was associated with a significant decrease in cancer incidence. Since then, half a dozen studies have confirmed it: Diabetics treated with metformin have from 25% to 40% less cancer than those who receive insulin as therapy or take sulfonylurea drugs that increase insulin secretion from the pancreas.

The idea that reducing insulin and insulin-like hormones in circulation may prevent tumors has become a bright hope for drug research. A host of insulin-suppressing drugs are in the pharmaceutical industry pipeline, says Lewis Cantley, director of the Cancer Center at Beth Israel Deaconess Medical Center, which is part of Harvard Medical School in Boston. But the companies may have been beaten to the punch: "Metformin may have already saved more people from cancer deaths than any drug in history," he says. It is one of the oldest and most commonly prescribed antidiabetic therapies in the world; some 120 million prescriptions are written for it yearly.

There is a caveat to the observational research linking metformin use to a decrease in cancer incidence, however: Studies of this kind are incapable of establishing a causal relationship. Maybe metformin prevents cancer in type 2 diabetics. Maybe insulin and the sulfonylurea drugs given instead of metformin promote cancer risk. Maybe something else entirely is going on.

Metformin activates an enzyme called AMPK in the liver, which then reduces the organ's synthesis and secretion of glucose, and thereby lowers blood glucose levels. But the drug also stimulates a tumor suppressor gene known as *LKB1*. Two University of Dundee biochemists, Dario Alessi and Grahame Hardie,

Dual use. Widely prescribed and cheap, metformin lowers insulin and, it appears, the risk of cancer.

worked out the AMPK-to-*LKB1* connection; Cantley and Reuben Shaw of the Salk Institute for Biological Studies in San Diego, California, did so independently. This connection was what prompted Morris to study cancer incidence in diabetics who are taking metformin.

As often happens in science, however, the physical mechanism may well be different from the hypothesized one. Instead of inhibiting cancer by activating AMPK and then *LKB1*, say Cantley and other researchers, metformin seems to work directly by lowering insulin and insulin-like growth factor (IGF) levels. "Metformin decreases glucose in the blood, and, as a secondary effect, decreases insulin levels," says Michael Pollak of McGill University in Montreal, Canada.

Evidence for that mechanism comes from animal studies. In September 2010, Phillip Dennis and his colleagues at the U.S. National Cancer Institute reported that metformin reduced lung cancer in mice that had been injected with potent tobacco-related carcinogens. But, as Dennis and his colleagues reported in the journal *Cancer Prevention Research*, they found no sign that metformin was activating AMPK in the lung tissue, as would be expected if that was the mechanism of action. In the liver, though, Dennis says, AMPK activation by metformin was "profound," and both insulin and IGF levels in the circulation were suppressed. The results, Cantley says, "support the hypothesis that anything that lowers insulin and IGF levels will inhibit tumor growth."

The results also "provide a strong rationale," as Dennis and his colleagues put it, for a clinical prevention trial. They hope to give metformin to patients who have had early stage lung cancer surgically removed and are at high risk of cancer recurrence. They intend to test the drug for safety, look for effects on insulin and IGF, and see whether it can prevent cancer in humans as it did for mice.

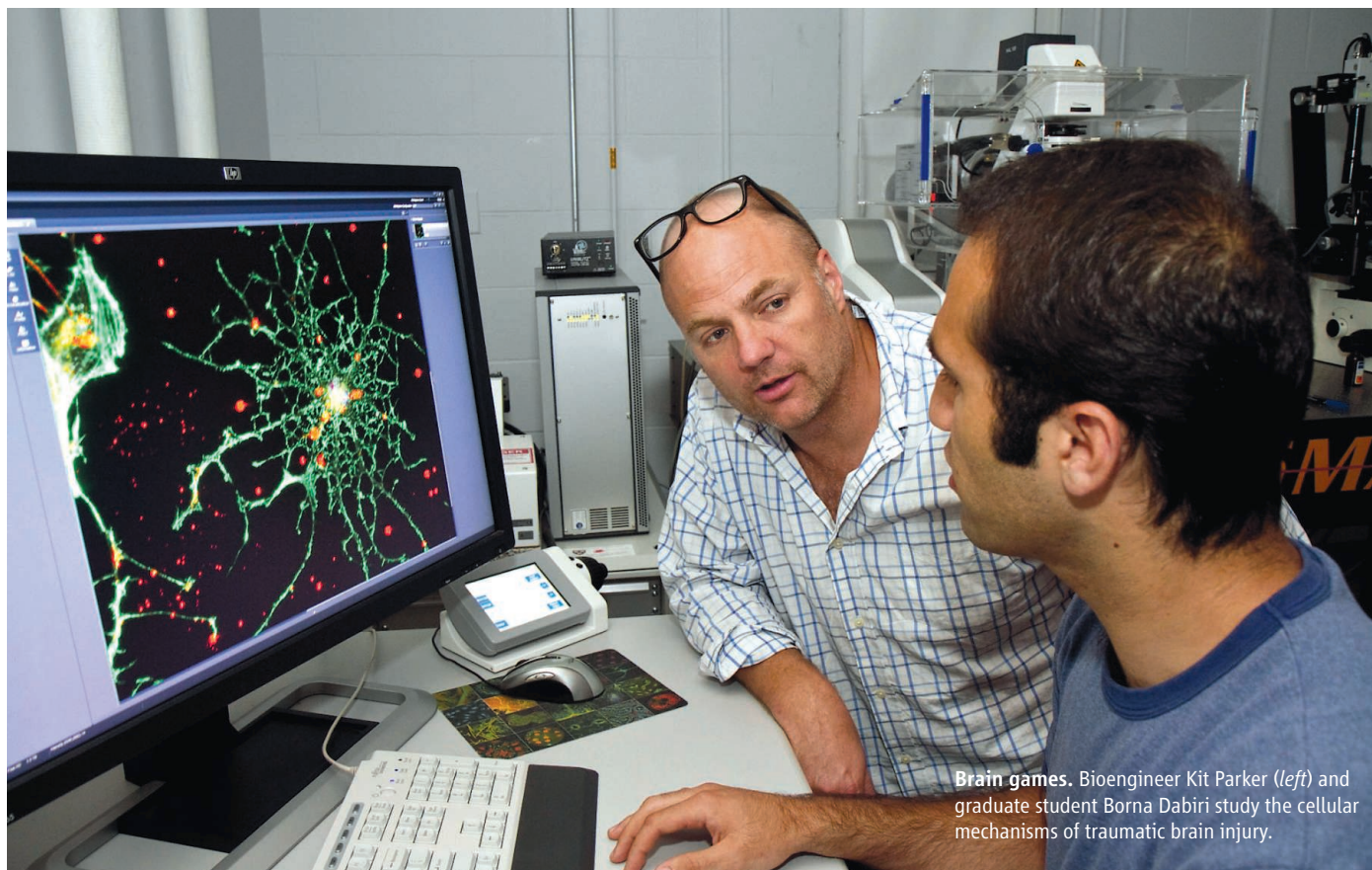
Other trials are beginning. One of the largest is being run by oncologist Pam Goodwin of the University of Toronto in Canada, who has been studying insulin and breast cancer since the mid-1990s. Goodwin says her interest was sparked after she realized that insulin may mediate the effects of obesity on breast cancer outcome. Fasting insulin levels in nondiabetic women are "predictive of breast cancer outcomes," she observes: Obese women have high insulin levels, and they "do badly."

Goodwin's group demonstrated 6 years ago that metformin lowers blood sugar and insulin levels by nearly one-quarter even in women who don't have diabetes. Now Goodwin and her colleagues are running a multinational clinical trial in which 3500 breast cancer patients will be randomized to receive either usual care plus a placebo or usual care plus metformin, to see if metformin helps improve survival and prevent recurrence of the disease. "We anticipate that it will take another 2 years to enroll everyone," she says, "and maybe 2 to 3 years after that before we have results."

Until then, as Goodwin emphasizes, the best they can say is that metformin *may* be beneficial for cancer. "All the preclinical and epidemiological evidence is pretty consistent and compelling, but all it's done is help us form a hypothesis. We need to proceed from here very, very carefully." **—G.T.**



Connecting the dots. Andrew Morris and colleagues showed that patients on metformin had 25% to 40% less cancer.



Brain games. Bioengineer Kit Parker (left) and graduate student Borna Dabiri study the cellular mechanisms of traumatic brain injury.

PROFILE: KIT PARKER

Engineering a New Line of Attack On a Signature War Injury

By jolting neurons in the lab, an Army officer and bioengineer hopes to gain ground on traumatic brain injury

When hijacked planes slammed into the World Trade Center towers in 2001, Kevin Kit Parker knew he had to do something. He'd always had a patriotic streak, and years earlier, while a graduate student in applied physics at Vanderbilt University in Nashville, Tennessee, Parker had enrolled in the Army Reserve Officers' Training Corps (ROTC). By the time of the attacks, he was a postdoctoral fellow, working on cardiac electrophysiology at Johns Hopkins University in Baltimore, Maryland, and in the middle of hunting for his first faculty position. He felt certain the country would soon be going to war, and despite having several job interviews on his calendar, he transferred to a unit he knew would be deployed. "I wanted to get in the game," he says.

While waiting to deploy, Parker accepted a job at Harvard University. With consider-

able trepidation, he asked the dean who'd just hired him for an immediate leave of absence to go to Afghanistan. It was a very unusual request, says then-dean Venkatesh Narayanamurti. Few, if any, Harvard professors have taken combat leave since World War II. But Narayanamurti admired Parker's dedication to national service. "I knew right away I would support him," he says.

By fall 2002, Parker was leading a team that patrolled a 900-square-kilometer swath between Kandahar and the Pakistan border, providing aid to villagers and searching for Taliban and Al Qaeda fighters. He finally started his job at Harvard in the summer of 2003, then deployed again in 2008, putting postdocs in charge of running the lab in his absence. His deployments caused Parker to reconsider the focus of his research and to establish a project on a signature injury of

the wars in Iraq and Afghanistan: traumatic brain injury (TBI). He has been back to Afghanistan twice more as part of a panel of experts convened to assess how the military handles TBI and combat stress.

The Pentagon estimates that more than 200,000 U.S. troops have experienced TBIs in the recent conflicts, mostly from roadside bombs and other improvised explosive devices (IEDs). The long-term effects of these brain injuries won't be known for decades, but there are already worrisome hints that TBI may compound the effects of combat stress and predispose veterans to the type of early-onset dementia seen in football players with a history of head injuries (*Science*, 29 July 2011, pp. 514 and 517). Despite the urgency of the problem, frustratingly little is known about the mechanisms by which an explosive blast injures the brain, Parker says. "I kept seeing guys get hit, and I thought, all right, I'll take a look at this and see if I can get a better angle on the problem."

Mission shift

On a recent morning, Parker's students and postdocs mill about a conference room before their weekly lab meeting. They pour coffee and set out a plate of jalapeño bagels

for Parker, who likes to goad others into eating spicy food. He arrives a few minutes late, wearing torn jeans and a red Harvard baseball cap with the bill folded into a sharp crease. A commanding presence at just under 6'6" (2 meters), Parker has a booming voice that bears more than a trace of his upbringing in west Tennessee. He launches into a list of lab business he's scribbled on a whiteboard. Some Italian researchers have asked about collaborating; so has a team from Merck, the pharmaceutical giant. And Parker has just returned from a molecular medicine conference in Korea. "Y'all make some damn fine fried chicken over there," he says to one of his Korean-born postdocs. "Hyungsuk, do you make that stuff at home?" When he shakes his head no, Parker pretends to be heartbroken. A second later, he's back to his list.

When Parker first arrived at Harvard, his main academic interest was the physical forces that determine how cells and tissues build themselves. His lab did cardiac tissue engineering, and that's still the focus for about two-thirds of his group. At the lab meeting, postdoc Anna Grosberg presents a computational tool she's developed for quantifying the alignment of sarcomeres, the protein fibers that make up muscle cells. How the fibers line up affects how a muscle contracts, and Parker thinks the tool could be useful for clinical pathologists or companies interested in engineering cardiac tissue for drug screens or therapies. In quick asides, he quizzes David Coon, who handles industry relations and intellectual property issues for the lab, about the commercialization prospects, and asks Sean Sheehy, a grad student with a computer science background, how hard it would be to incorporate Grosberg's metric into a graphical software package. When Sheehy says it's doable, Parker jokingly tells him: "This is your project now, baby!"

Ideas and projects spring up freely in the lab. A cotton-candy machine inspired a new way for making nanofiber scaffolds on which to grow cells (and a 2010 paper in *Nano Letters*). Back in his office, Parker shows off a movie on his computer of a more recent project: an artificial jellyfish. Cut from a polymer sheet coated with rat heart muscle cells, its form lacks the organic curves of the real thing, but the ghostly flap

of tissue pulses across the screen with surprisingly lifelike motion.

Parker sees his fledgling TBI research project as a moral obligation. He saw IED explosions firsthand in Afghanistan, and he has buddies who've suffered the consequences. When Colonel Geoffrey Ling, the program manager who oversees TBI research at the Defense Advanced Research Projects Agency (DARPA), asked Parker in 2006 if he'd ever thought about studying TBI, he demurred at first. "I said, 'There have to be better people than me; I'm not a brain guy,'" Parker says. But as he started reading the scientific literature, he was struck by how little was known about what happens at the cellular level in a TBI.



In the thick of it. Parker, here searching for IEDs in Afghanistan, has changed the course of his research after two tours of duty.

Concussion on a chip

One prevalent idea has been that a blast wave or physical blow to the head tears the membranes of neurons, allowing positive ions to rush in and overexcite neurons to the point of killing them. Based on his experience with tissue engineering, Parker suspected something else might be going on instead, or in addition. He was surprised to see nothing in the research literature about integrins, proteins in the membrane of all cells that connect a cell's internal protein skeleton to the scaffold of proteins outside the cell, the so-called extracellular matrix. Parker reasoned that the force of a blast could propagate through this network of proteins, interfering with integrins and the many cell-signaling pathways they interact with.

The first challenge was figuring out how to go about studying TBI in the lab. Researchers have studied TBI by issuing blows to the heads of rats, pigs, and other animals, but it's not clear how well those experiments replicate what the human brain experiences in a car crash or explosive blast. Moreover, Parker says, "if I start blowing up goats at Harvard, I'm not going to last long." As an alternative, his lab has devised an arsenal of devices that can subject cultured neurons or slices of brain tissue to carefully calibrated forces. "We need to think of ways to replicate this on the bench top so you can mainstream the science," Parker says.

Their early work supports the idea that integrins may play a role in TBI. In one study, graduate student Matthew Hemphill and others put cultured rat neurons on a stretchy, square sheet of silicone that could be given a short tug by a high-precision motor. These tugs subjected the neurons to forces that the researchers estimated would be similar to those generated inside the head of a soldier exposed to an IED blast. Within a few minutes, microscopic swellings appeared on the spindly axons and dendrites that send and receive messages from neighboring neurons. Axonal injury is a hallmark of TBI, and a diffusion tensor imaging study by a different group published 2 June 2011 in *The New England Journal of Medicine* found evidence of axon damage in U.S. soldiers who suffered TBIs in Iraq. Additional experiments with the cultured rat neurons implicated a particular

integrin signaling pathway in this damage. Treating the neurons with a drug that inhibits a component of this pathway called Rho-kinase reduced damage to neurons after a simulated blast, the researchers reported in *PLoS ONE* in July 2011.

In another study, published 2 August 2011 in the *Proceedings of the National Academy of Sciences*, a team led by then-postdoc Patrick Alford used the same setup to investigate the effects of a simulated blast on blood vessels. In this case, the researchers used rat muscle cells from the lining of blood vessels. When subjected to a sudden stretch, these cells flipped a genetic switch that made them more likely to contract and promoted their proliferation. Both effects would tend to clamp down on blood vessels,

which could exacerbate a brain injury by depriving injured neurons of oxygen, Parker says. Again, inhibiting Rho-kinase reduced these effects.

The findings could help clarify something that has baffled clinicians, says Jack Tsao, a neurologist and neuroscientist with the U.S. Navy and the Uniformed Services University of the Health Sciences in Bethesda, Maryland. Blood vessels often constrict after a ruptured aneurysm or severe head injury causes bleeding into the brain. This response, called vasospasm, can cause a stroke by cutting off the blood supply to areas away from the injury. But Tsao says some service members with TBIs exhibit strokelike symptoms and other evidence of vasospasm even when brain scans show no signs of bleeding. The integrin mechanism Parker's team identified might explain how vasospasm can occur in the absence of bleeding.

"These are both very elegant papers," says David Hovda, a neuroscientist and director of the Brain Injury Research Center at the University of California (UC), Los Angeles. They highlight a potential mechanism of TBI that hasn't been considered before, Hovda says, but one that makes a lot of sense and raises interesting possibilities for treating TBI or minimizing its effects with drugs.

On a recent visit, Hemphill and lab engineer Josue Goss, an Iraq War veteran himself, demonstrated a "blast bio-reactor" that compresses neurons instead of stretching them. An aluminum contraption with a piston, it delivers a quick punch to cells sandwiched between two layers of gel. As a shock wave from a blast propagates through the brain, neurons may be subjected to this kind of punishment in addition to stretching, Hemphill says.

Parker now wants to recruit three postdocs with neuroscience backgrounds to help expand his TBI research. One ambitious plan is to construct an amygdala on a chip. The amygdala is a hub in the brain's emotional circuitry, and the region has been implicated in human brain imaging studies of post-traumatic stress disorder. Parker wants to grow amygdala neurons on a chip and subject them to simulated blasts to see whether they're more sensitive to injury than neurons in other parts of the brain.

Parker's work on TBI led to an invitation to join the "gray team," a panel of experts

convened by Admiral Mike Mullen, then chair of the Joint Chiefs of Staff, to advise the military on issues related to TBI and combat stress. Parker traveled to Afghanistan with other team members twice in 2011. They visited a DARPA field station that is testing blast dosimeters to measure the forces troops experience in an explosion, and they also studied the logistics of bringing an MRI scanner to Afghanistan so neurologists can get images of soldiers' brains within hours of a TBI rather than days.

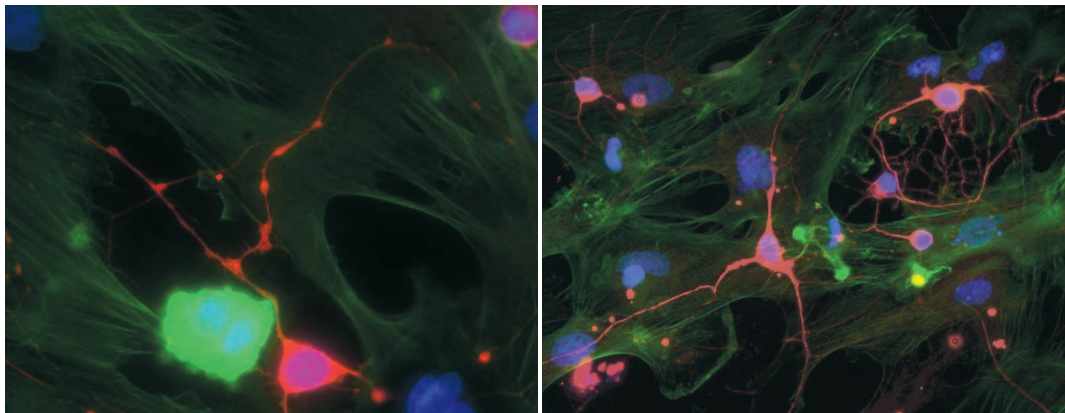
Postdocs in command

Parker's absences have thrust the young scientists in his lab into leadership roles early in their careers. His second deployment to Afghanistan in December 2008 lasted 8 months. He charged three of his most senior postdocs—Alford, Grosberg, and Adam Feinberg, who is now an assistant professor at Carnegie Mellon University in

UC Irvine this spring. "I have a better idea what I'm facing," she says.

Parker says he tries to prepare his postdocs for the next stage in their career by pushing them to take on increasing responsibility for research decisions and mentoring grad students: "In the military, you're training all the time; you're developing your subordinate leaders." His military training influences the lab in other ways, too, from the meticulously clean workspaces to the uniformity of the font in PowerPoint slides. ("We're Arial all the way," says Megan McCain, a graduate student. "If you used Times New Roman there could be trouble.")

Parker acknowledges that his style is not for everyone, and not everyone who has joined the lab has stuck around as long as planned. But he talks passionately about the obligation he feels toward those who do. He's taken several veterans of the Iraq and Afghanistan wars into his lab. "If you give



Blast damage. Neurons (red label) subjected to a simulated blast (*left*) exhibit swellings on their axons and dendrites, a sign of damage that's absent in healthy neurons (*right*).

Pittsburgh, Pennsylvania—with overseeing a long list of projects and collaborations and \$2.5 million in grants. Parker got in touch when he could by cell phone, about once a month. "Sometimes we could hear helicopters in the background," says Feinberg, who was the person in overall charge. As a group, the postdocs managed to keep the lab running more or less smoothly, Feinberg says. "We would go to breakfast once a week and hammer out what was going on with different projects, keeping track of grad students' progress and things we needed to get done for grants [and] paper writing."

Feinberg says the biggest challenge was keeping his own research on track: "My bench work suffered." But he says the experience served him well in getting a job and setting up his own lab. Alford started a job at the University of Minnesota, Twin Cities, last year, and Grosberg will begin a job at

them something to do, these kids will not stop," he says. "It raises the professionalism of the whole lab."

Now 45, Parker has a wife and young daughter, and an ever-growing list of responsibilities on and off campus. He played an active role in bringing ROTC back to Harvard after a 40-year absence dating back to the Vietnam War, for instance, and now serves as the campus coordinator. His lab is running at full speed, and he's eager to see the TBI project take off. He doesn't relish the thought of leaving his family or getting shot at again, but he says he needs a sense of closure on the war in Afghanistan, and perhaps, too, on a chapter of his life in which he craved more action and adventure than the academic lifestyle typically allows. When the mission in Afghanistan finally wraps up, he says, he wants to be there to see it.

—GREG MILLER



LETTERS

edited by Jennifer Sills

NextGenVOICES

Results: Future of a Generation

In October, we called on young scientists to answer these questions: How will the practice of science change in your lifetime? What will improve and what new challenges will emerge?

We received more than 100 responses from young scientists across the globe. Although concerns about the future are evident, these scientists spoke passionately about meeting the challenges they face and embracing science's potential to address the world's problems. We've included a sample of the best responses here (ellipses indicate excerpted text). To read more, go to <http://scim.ag/NextGenResults>.

Submit Now: Definition of Success

Would you like to make your voice heard in *Science*? Our second NextGen VOICES survey is now open. We would like to hear your answer to these questions:

What is your definition of a successful scientist? How has this definition changed between your mentor's generation and your own?

To submit, go to http://scim.ag/NextGen_2.

Deadline for submissions is 17 February. A selection of the best responses will be published in the 6 April issue of *Science*. Submissions should be 250 words or less. Anonymous submissions will not be considered. Please submit only once.

was perpetuated by a tremendous pressure to perform and succeed. Deep-thinking spaces were eroded by the immediacy of communication, and swathes of information and data. Science and technology have assisted with nature-mimicking algorithms to respond



to pedestrian tasks like sifting through e-mail. Virtual connectedness increased international, cross-institutional, and multidisciplinary research with the merging of our social and professional lives, but scientists continued to grapple with disconnected "silo" mentalities and the natural-social science divide. Self-preservation continued

to drive research institute agendas. The rise of "super-universities" at the edge of the open-source revolution was always on the horizon. Scientists were so busy that they were often blind to what their universities were morphing into, although things have started to change recently. There have been some major successes to celebrate, such as the use of desiccation-resistant gene technology for plants (developed in South Africa), which turned many African deserts into productive farmland. Like the first heart transplant of the previous century, it shows that Africa has much to contribute.

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THE BIGGEST CHALLENGE FACING A GENERATION of young scientists is breaking free of the shackles placed on them by their predecessors. We are tasked with fixing large dysfunctional institutions that we are given no authority over nor trained to take the reins of. We are expected to fix broken peer-review systems riddled with small insular cliques.

NextGen Speaks

...TODAY, UNRAVELING NEW PRINCIPLES OF any system or process typically demands several smaller steps, each warranting publication.



The future practice of science will traverse smaller steps in parallel, through online cooperation between experts, each tackling smaller steps while continuously communicating findings to their collaborators.

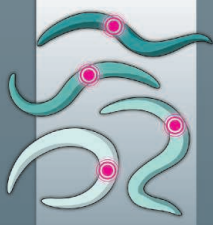
This may be envisioned as skipping the stairs and, rather, taking an elevator. The result? Each paper will contain fewer questions and more answers. If communication truly erases distance in the scientific community, new fields are likely to emerge in the intersections between disciplines. Bridging the gaps between disciplines such as neuroscience and psychology is one of the triumphs I personally hope to see happen.

Again, communication is the key, and it may not come easily. The sharing of knowledge in a competitive world is perhaps the greatest challenge for we who are the next generation of scientists.

ASGEIR KOBRO-FLATMOEN

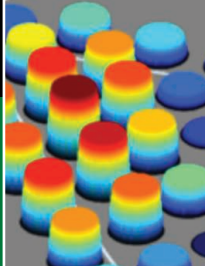
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OUR RETIREMENT SPEECH, IN 2045: DURING the first part of this century, the economic recession burned into the collective memory. Society demanded better "value for money" from its scientists, requiring them to demonstrate the impact of their research on global and national challenges. Some of those challenges, such as climate change and resource depletion, have only partially been met as scientists have struggled to adapt in a rapidly changing world. Academics spent too much time doing and too little time thinking; this



Influencing
mutation effects

44



View of fields

47

We are expected not only to flourish in, but to be thankful for a funding structure equipped with scarce resources that are primarily used on contract science for the blessed few rather than discovery based on the merits of ideas and early results. We are expected to survive and contribute to a larger society that no longer trusts scientists, appreciates expertise, or

understands the value science brings to the world. Science will continue to be dictated from above, not practiced. Science will be performed by those who are willing, not those best prepared or suited

to the task. This future will be brought about in part because of disturbingly low levels of support in our schools for science and math education, spiraling the globe toward all-time lows in scientific literacy during the modern era. This is the future young scientists see ahead of us—a future handed down by a generation who was more interested in glorifying themselves than leaving things better than how they found them. We are expected to rise to this challenge and we're sure as hell going to give it our all. Gosh, who needs a drink?

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AS WE ACQUIRE KNOWLEDGE, WE CLIMB A LADDER that helps us to see farther and observe unexplored territory. Our predecessors made enormous contributions while climbing the ladders of reductionism. As they climbed, they also became more isolated. The distance between disciplines and subdisci-



plines increased, as they focused on their own research topics. The challenge today is how to connect those ladders. To build taller ladders of knowledge, we have to create bridges among disciplines and not be afraid of the distance to the ground. It is, however, unrealistic to think that single individuals can master several disciplines. A side effect of increasing interdisciplinarity would therefore be an increase in the number of co-authors of scientific publications. Scientists, even more than today, will have to learn how to work together. Because of the current vast amount of knowledge, eclectic research groups will have to play the role that a single scientist played in the 19th century. To facilitate relationships between disciplines, the way we communicate our results among ourselves, and to the society, has to evolve. Scientific communication will be transformed into a multimedia experience. Live talk repositories, animations, and videos will enhance the figures of papers as unique pieces of scientific knowledge. We are therefore entering the Era of Interdisciplinarity. It is the end of many ivory towers. It is the beginning of many bridges.

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SCIENCE IS BECOMING INCREASINGLY ACCESSIBLE to minorities, women, and people from a variety of cultural and socioeconomic back-



grounds. Because more people are now being exposed to science, I predict that the speed and significance of scientific advancements will increase dramatically over the next 100 years. My grandmother was an extremely intelligent woman who spoke five languages, but she left high school before receiving her degree so that she could work in a mill to support her family. I wonder what contributions she could have made to the world of science, or any other field, if she had lived in a time of gender equality and financial aid. Today, great minds of all races, religions, and sexes are gathering in universities to collaborate on research and approach problems with their own unique perspectives....

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... A NEW CAREER PATH IN SCIENCE will emerge. The professional scientific data manager will have a unique skill set from areas such as statistics, large database administration, and information design....



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UBIQUITY OF TABLET TECHNOLOGY will result in near-real-time textbook updates. School curricula will therefore need to change on a daily basis....

MICHAEL YOUNG

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... WITH THE RISE OF TECHNO-ENTREPRENEURSHIP among researchers, science will soon encompass more of other disciplines such as business, marketing, and intellectual property. Collaborative groups will successfully create spinoffs and fund each other's research. A couple of mini-MBAs and certificate courses in finance will align themselves along with scientific publications in a researcher's resume....

KINGSHUK PODDAR

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THE COST OF PLASTIC-BASED materials will continue to rise as fossil fuels run dry, but the knowledge to work with glass has faded. When Corning no longer makes disposable, plastic everything, I think our lab will have to shut down....

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AS A 16-YEAR-OLD MAKING MY FIRST STEPS in the world of research, I fear that it will be hard to find interesting phenomena to study. It seems that most of the major scientific questions have been answered, or will be in the near future. In the past, the major obstacle to vast scientific progress was technology. I'm concerned that few science-catalyzing technological advancements are forthcoming. ... Nonetheless, the abundance of research tools presents a big upside: When a scientist from my generation finds a research subject,



they will most likely have the instruments needed, instruments that past generations could only dream of.

OR SAGY

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THE KEY THEME DISTINGUISHING the future practice of science will be integration. As access to information reaches unprecedented levels, the ability to interpret information into meaningful patterns will become much more complicated. In this rigorous intellectual environment, collaboration will be critical for scientific discovery. The geologist will contact the sociologist. The psychologist will share a coffee with the physicist. The next generation of scientists will also experience a stronger union (or reunion) between science and the arts. Scientists will find a useful medium in artistic expression for visualizing information in innovative ways that explain fundamental processes in nature. Statistical models will transform in moving mobiles. Two-



dimensional graphs will be replaced by three-dimensional sculptures. The canny work of Nathalie Miebach illustrates the useful role that art can play in science. Her musical and visual manifestations of meteorological data are not only remarkably creative, but also reveal obscure relationships underlying the dynamics of weather systems. The preeminent scientific questions of the future will not be answered through convention and self-preservation. Rather, the most influential insights will be gained through integration, artistic expression, and openness.

DAVID R. DAVERSA

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... WITH IMPROVEMENTS TO data tagging, the Semantic Web, and evolution of Google Scholar, raw data will be published and social science findings will no longer be discovered by individual researchers, but instead validated through simultaneous tests among myriad samples collected by many organizations and researchers, all of whom use a common semantic data-tagging system....



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I THINK, IN MY LIFETIME, SCIENCE WILL BECOME more open, accessible, and "democratic."



People will have more opportunity to make small contributions in doing big projects. Monopoly of big money and secretive research will break, and the benefits of the latest scientific research will actually

reach common people.... **ASHUTOSH GUPTA**

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CORRECTIONS AND CLARIFICATIONS

News & Analysis: "Berkeley engineering students demand greater effort to promote diversity" by J. Mervis (2 December 2011, p. 1191). *Science* incorrectly calculated the percentage of underrepresented minorities (URMs) in the freshmen engineering class depicted in the bar chart for the University of California, Los Angeles (UCLA). It should be 10.2%. The number shown for URMs, 47, is correct.

News & Analysis: "Curious cosmic speed-up nabs Nobel Prize" by A. Cho (7 October 2011, p. 30). The last line of the story states that Edwin Hubble discovered that the universe is expanding. In fact, Georges Lemaître made this discovery before Hubble.

TECHNICAL COMMENT ABSTRACTS

Comment on "Nonreciprocal Light Propagation in a Silicon Photonic Circuit"

Shanhui Fan, Roel Baets, Alexander Petrov, Zongfu Yu, John D. Joannopoulos, Wolfgang Freude, Andrea Melloni, Miloš Popović, Mathias Vanwolleghem, Dirk Jalas, Manfred Eich, Michael Krause, Hagen Renner, Ernst Brinkmeyer, Christopher R. Doerr

We show that the structure demonstrated by Feng *et al.* (Reports, 5 August 2011, p. 729) cannot enable optical isolation because it possesses a symmetric scattering matrix. Moreover, one cannot construct an optical isolator by incorporating this structure into any system as long as the system is linear and time-independent and is described by materials with a scalar dielectric function.

Full text at www.sciencemag.org/cgi/content/full/335/6064/38-b

Response to Comment on "Nonreciprocal Light Propagation in a Silicon Photonic Circuit"

Liang Feng, Maurice Ayache, Jingqing Huang, Ye-Long Xu, Ming-Hui Lu, Yan-Feng Chen, Yeshiahu Fainman, Axel Scherer

Fan *et al.* raised technical concerns about our study regarding the Lorentz reciprocity theorem, with which we completely agree. Unfortunately, we incorrectly used the term "nonreciprocal" to describe the behavior of electromagnetic propagation in our devices. In our paper, this term does not refer to Lorentz reciprocity but to the asymmetric mode conversion that is experimentally demonstrated.

Full text at www.sciencemag.org/cgi/content/full/335/6064/38-c

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

Response to Comment on “Nonreciprocal Light Propagation in a Silicon Photonic Circuit”

Liang Feng,^{1,2,4*} Maurice Ayache,³ Jingqing Huang,^{1,4} Ye-Long Xu,² Ming-Hui Lu,²
Yan-Feng Chen,² Yashaiahu Fainman,³ Axel Scherer^{1,4}

Fan *et al.* raised technical concerns about our study regarding the Lorentz reciprocity theorem, with which we completely agree. Unfortunately, we incorrectly used the term “nonreciprocal” to describe the behavior of electromagnetic propagation in our devices. In our paper, this term does not refer to Lorentz reciprocity but to the asymmetric mode conversion that is experimentally demonstrated.

We recently demonstrated a silicon (Si) waveguide-based device that performs mode conversion in only one direction (*I*). We called this effect nonreciprocal, a term that could be misleading as it implies that our device avoids Lorentz reciprocity. Indeed, either a magnetic or a time-dependent response is needed to convert our device into an optical isolator. An analysis of reciprocity of our asymmetric optical mode converter, obtained by deriving the corresponding scattering matrix, confirms that the mode conversion of our device is not sufficient for optical isolation on its own. Nevertheless, we believe, it may provide a useful element for on-chip optical isolation in Si photonics when combined with nonlinear optical wave mixing. Even though our device is not adequate to provide optical isolation, we have demonstrated spontaneous breaking of parity-time symmetry.

Our device uses a complex variation in the effective dielectric constant to generate an asymmetric wave vector q . Reciprocity can be assessed by deriving and analyzing the corresponding scattering matrix by solving the coupled mode equations in (*I*). It can be shown that at the two ends of the device in the z direction, $z = 0$ and $z = L/2$ ($L = 2\pi/q$ is the period of modulations), the fields are as follows:

$$A_1\left(\frac{L}{2}\right) = \exp\left(-\frac{B_1}{\pi}L\right)A_1(0) + iC \exp\left(-\frac{B_1+B_2}{2\pi}L\right)$$

$$\begin{aligned} & \times \int_0^{L/2} \exp\left(\frac{B_2-B_1}{2\pi}L\left(\exp\left(i\frac{2\pi z}{L}\right)\right)\right) dz A_2(0) \\ A_2\left(\frac{L}{2}\right) &= \exp\left(-\frac{B_2}{\pi}L\right)A_2(0) \\ A_{-1}(0) &= \exp\left(-\frac{B_1}{\pi}L\right)A_{-1}\left(\frac{L}{2}\right) \\ A_{-2}(0) &= \exp\left(-\frac{B_2}{\pi}L\right)A_{-2}\left(\frac{L}{2}\right) + \\ & iC \exp\left(-\frac{B_1+B_2}{2\pi}L\right) \\ & \times \int_0^{L/2} \exp\left(\frac{B_2-B_1}{2\pi}L\left(\exp\left(i\frac{2\pi z}{L}\right)\right)\right) \\ & \times dz A_{-1}\left(\frac{L}{2}\right) \end{aligned} \quad (1)$$

where A_n corresponds to the field strength and B_n is the mode integral for mode n , with positive or negative n indicating forward or backward propagation, respectively. The coefficients C gives the modal power overlap for modes 1 and 2 (i.e., symmetric and antisymmetric modes, respectively), with $C = C_1 = C_2$. If we treat the first and second modes, for both forward and backward propagation, as a four-port system with the two ends of the device as input and output, the above equations yield the following scattering matrix:

$$\begin{bmatrix} A_{-1}(0) \\ A_1\left(\frac{L}{2}\right) \\ A_{-2}(0) \\ A_2\left(\frac{L}{2}\right) \end{bmatrix} = S \begin{bmatrix} A_1(0) \\ A_{-1}\left(\frac{L}{2}\right) \\ A_2(0) \\ A_{-2}\left(\frac{L}{2}\right) \end{bmatrix}$$

$$= \begin{bmatrix} S_{11} & S_{12} & S_{13} & S_{14} \\ S_{21} & S_{22} & S_{23} & S_{24} \\ S_{31} & S_{32} & S_{33} & S_{34} \\ S_{41} & S_{42} & S_{43} & S_{44} \end{bmatrix} \begin{bmatrix} A_1(0) \\ A_{-1}\left(\frac{L}{2}\right) \\ A_2(0) \\ A_{-2}\left(\frac{L}{2}\right) \end{bmatrix} \quad (2)$$

where

$$S = \begin{bmatrix} 0 & \exp\left(-\frac{B_1}{\pi}L\right) & 0 & 0 \\ \exp\left(-\frac{B_1}{\pi}L\right) & 0 & 0 & 0 \\ 0 & iC \exp\left(-\frac{B_1+B_2}{2\pi}L\right) \int_0^{L/2} \exp\left(\frac{B_2-B_1}{2\pi}L\left(\exp\left(i\frac{2\pi z}{L}\right)\right)\right) dz & 0 & 0 \\ 0 & 0 & 0 & \exp\left(-\frac{B_2}{\pi}L\right) \end{bmatrix}$$

This scattering matrix S is symmetric, indicating that the system is reciprocal (2). Consequently, the transmission of both modes is the same for both directions, which has been shown in fig. S1 in (*I*) and by Fan *et al.* (2). Moreover, because $S_{32} \neq S_{41}$, the device demonstrated in (*I*) converts the first mode to the second mode only in one direction. The exponential elements in the matrix show that power decays as light propagates because the device introduces loss due to metals.

Nevertheless, the demonstrated asymmetric mode conversion, combined with the inherent optical nonlinearity of Si, may still be useful to achieve nonreciprocal transmission in a Si waveguide on-chip without integrating other complementary metal-oxide semiconductor-incompatible materials. For example, the antisymmetric mode can be designed to be necessary for the phase matching of the third harmonic generation ($k^{3\omega} = 2k_1^\omega + k_2^\omega$). Improvement of nonlinear efficiency using a slow-light structure (3) and vanishing two-photon absorption in Si (4) might be important to achieve distinguishable transmission in different directions (5).

In summary, the one-way mode converter is Lorentz reciprocal and on its own cannot be used as the basis of an optical isolator. It is also lossy [7 dB for the power in the symmetric mode in (*I*)] and will require gain to be added in order to be useful. Similar optical modulation approaches using exponential functions were theoretically proposed by Greenberg and Orenstein (6, 7), which were not included in our original reference list. We thank Fan *et al.* (2) for clarifying our work and helping to avoid misunderstanding.

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Comment on “Nonreciprocal Light Propagation in a Silicon Photonic Circuit”

Shanhui Fan,^{1*} Roel Baets,^{2,3*} Alexander Petrov,^{4*} Zongfu Yu,¹ John D. Joannopoulos,⁵ Wolfgang Freude,^{6,7} Andrea Melloni,⁸ Miloš Popović,⁹ Mathias Vanwolleghem,^{10,11} Dirk Jalas,⁴ Manfred Eich,⁴ Michael Krause,¹² Hagen Renner,¹² Ernst Brinkmeyer,¹² Christopher R. Doerr¹³

We show that the structure demonstrated by Feng *et al.* (Reports, 5 August 2011, p. 729) cannot enable optical isolation because it possesses a symmetric scattering matrix. Moreover, one cannot construct an optical isolator by incorporating this structure into any system as long as the system is linear and time-independent and is described by materials with a scalar dielectric function.

In (1), Feng *et al.* consider a two-mode waveguide with a spatially varying but time-independent dielectric constant (Fig. 1), and numerically and experimentally demonstrate a one-way modal conversion effect: The odd mode is strongly excited when an even mode is incident along the backward direction from the right end of the waveguide (Fig. 1b). In contrast, the odd mode is not excited when the same even mode is incident along the forward direction from the left end (Fig. 1A). Related to (1), we note that a similar one-way modal conversion effect, with the same pattern of spatially varying dielectric constant, was previously considered theoretically in (2, 3).

The results in (1) have generated widespread interest. Based on the results, Feng *et al.* claim the existence of nonreciprocity in their design and suggest the possibilities of applying the observed effect toward creating on-chip optical isolators. However, one must ask: (i) Is the observed effect of one-way photonic modal conversion truly a proof of optical nonreciprocity? (ii) Can

the structure in (1) indeed be used toward creating an optical isolator?

Unfortunately, the answers to both of the questions above are “no.” The structure in (1) is in fact reciprocal. As a result, one cannot construct an optical isolator this way.

It is well known that any time-independent linear system, described by a symmetric electric permittivity tensor $\epsilon(r)$ and a symmetric magnetic permeability tensor $\mu(r)$, is constrained by the Lorentz reciprocity theorem (4–6). Such a system is reciprocal in the sense that its scattering matrix is symmetric (4–6). Very importantly, the reciprocity theorem applies even when $\epsilon(r)$ or $\mu(r)$ is complex, that is, even when the system has gain or loss.

The experimental structure studied in (1) consists of silicon, silicon dioxide, germanium, and chromium. No magnetic field is applied. All these materials are known to have symmetric permittivity tensors (and diagonal permeability tensors), and indeed in (1) both $\epsilon(r)$ and $\mu(r)$ are treated as scalars theoretically. Thus, the structure in (1) is subject to the reciprocity theorem and has a symmetric scattering matrix.

In Fig. 1, A and B [which are equivalent to fig. S1 in the SOM of (1)], one injects the even mode along either forward or backward directions. Notice that the power transmission coefficients T_{ee} to the even mode (red curves in Fig. 1, A and B) are the same for both propagation directions for any given length d of the spatially varying region, as expected from the reciprocity theorem. Likewise, the power transmission coefficient T_{oe} to an odd mode, when an even mode is injected from the right (blue curve in Fig. 1B), is the same as the power transmission coefficient T_{eo} to an even mode, when an odd mode is injected from the left (red curve in Fig. 1C).

Why doesn’t the structure in (1) function as an optical isolator? Certainly, in this structure, the even mode injected from the left is strongly attenuated without exciting the odd mode (Fig. 1A), whereas the even mode injected from the right

excites the odd mode and hence has a substantial power transmission coefficient T_{oe} (Fig. 1B). Thus, there appears to be a contrast between the two propagation directions. However, one should not confuse such a contrast with what is required of an optical isolator. An optical isolator needs to suppress back-reflection irrespective of modal content. A simple way to suppress back-reflection is to attenuate it, but in reciprocal structures this implies, unfortunately, that the forward light is also attenuated. The only way to have a good transmission for the forward propagating light and to simultaneously suppress the back-reflection is to use a device in which at least one trans-

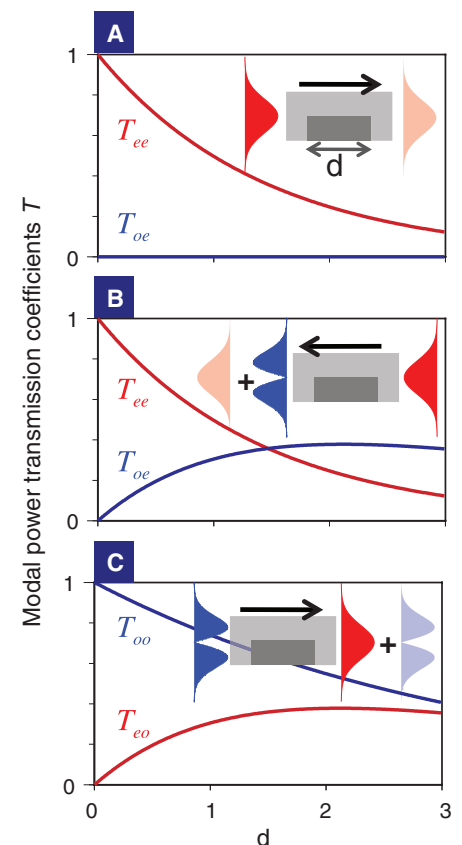


Fig. 1. Modal power transmission coefficients in a waveguide (gray region in the insets) supporting an even and an odd mode, as a function of length d of a region (dark gray region in the insets) in the waveguide where the dielectric constant varies with a profile $\delta\epsilon \propto \exp(+ikx)$, with x being along the axis of the waveguide. Red and blue curves are transmission coefficients to even or odd modes, respectively. In the inset, the light gray regions have a real dielectric constant. The bold arrows indicate the propagation directions. (A) Even mode injected from the left. Power transmission coefficient T_{ee} from even to even mode and T_{oe} from even to odd mode. (B) Even mode injected from the right. Power transmission coefficients T_{ee} from even to even mode and T_{oe} from even to odd mode. (C) Odd mode injected from the left. Power transmission coefficients T_{oo} from odd to odd mode, and T_{eo} from odd to even mode.

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mission from a given input mode to a given output mode is asymmetric, that is, different for forward and backward propagation direction. In the structure in (I), however, all mode-to-mode transmissions are symmetric. Consequently, light reflected back into the odd mode at the left end will necessarily pass through the structure with the same high power transmission coefficient T_{eo} , equal to T_{oe} (Fig. 1C, red curve). Therefore, the structure cannot function as a part of an isolator: It has equal backward and forward transmission coefficients between any two modes of the system.

More generally, one cannot construct an optical isolator with any structure having a symmetric scattering matrix. Therefore, one cannot construct an optical isolator by incorporating the structure of Feng *et al.* into any system containing any combination of components or signal processing elements as long as the overall system is linear and time-independent and is described by materials with a scalar dielectric function.

To summarize, the structure in (I), which is linear and time-independent, is reciprocal in the sense that it has a symmetric scattering matrix, which fundamentally differentiates it from optical isolator structures, including magneto-optical isolators, as well as nonlinear (7, 8) or time-dependent structures (9, 10) where reciprocities are broken. One cannot construct an optical isolator by simply enclosing the structure in (I) with a system containing any combinations of components or signal processing elements, as long as the overall system is linear and time-independent and is constructed only from materials with symmetric permittivity and permeability tensors. To achieve true optical isolation, the scattering matrix of the system must be nonsymmetric.

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MOLECULAR BIOLOGY

RNA Rejoice!

Joan A. Steitz

In the beginning, there was RNA. But, as in Genesis, the telling is what brings the story to life. No one could be better suited to trace the history of “life’s indispensable molecule” than James Darnell. It was he who discovered RNA processing shortly after the centrality of RNA in all living systems was established by the identification of messenger RNA in 1961. His lab gathered the first evidence that the useful form of most RNAs—unlike those of DNA and proteins—is fashioned by trimming and modifying the originally synthesized molecule in predetermined ways. Darnell’s recent seminal contributions to our understanding of mechanisms of transcriptional regulation extend his panoramic view of the versatility of RNA.

The thread that stitches *RNA: Life’s Indispensable Molecule* together is the perhaps obvious concept that RNA determines a cell’s functioning. DNA may encode a cell’s potential, but the RNA molecules present dictate the activities that define a cell’s state at any particular moment.

Darnell begins his account by reaching back to the days before the revelation of the structure of DNA and describing the emergence of the concept (amid significant resistance) that linear macromolecules orchestrate information flow within cells. The proof that proteins are long arrays of amino acids linked in definite order came first. After 1953, DNA’s elegant double-stranded structure convinced most scientists that it must contain the corresponding linear genetic information. But how were proteins connected to genes? Darnell next focuses on the steps culminating in the discovery of messenger RNA, which firmly fixed the role of RNA as the central player.

The book then builds the RNA story era upon era. The explosion during the 1960s and 1970s in fundamental understanding of how RNA and protein synthesis are regulated in bacteria, including deciphering of

the genetic code, sets the stage for the two lengthy chapters that form the centerpiece of the book. Switching to mammalian cells, Darnell begins by describing the critical but painstaking progress of investigators who coaxed animal cells (including HeLa cells) and their viruses to grow in culture. These advances enabled the completely unanticipated discovery of splicing, as well as elucidation of the basic principles of transcription that differ in eukaryotic cells compared to bacteria. The monumental chapter “Controlling mRNA” spans 1980 to the present, including—in palatable form—the factorology of transcription and alternative splicing before finishing off with microRNAs and small interfering RNAs. Here Darnell provides clearer insights

than I have found elsewhere into the relationship between chromatin modifications and the initiation and elongation of RNA chains. Often, he steps back to look at the prevailing assumptions of the time and how they set the stage for the next critical

advance. Even the last chapter, on evolution, which considers the many types of cellular RNA molecules that continue to emerge, says just enough to convince the reader that exploring RNA will continue to offer surprises and revelations.

Particularly worth savoring is Darnell’s rendition, based on firsthand knowledge, of the confusion that reigned in the early 1970s regarding the relationship between nuclear and cytoplasmic RNA. It had been known since the late 1950s that not all the RNA synthesized in the nucleus of animal cells emerges into the cytoplasm. The existence of very large nuclear molecules called heterogeneous nuclear RNA (hnRNA) was documented (primarily by Darnell) 14 years before the discovery of introns in pre-

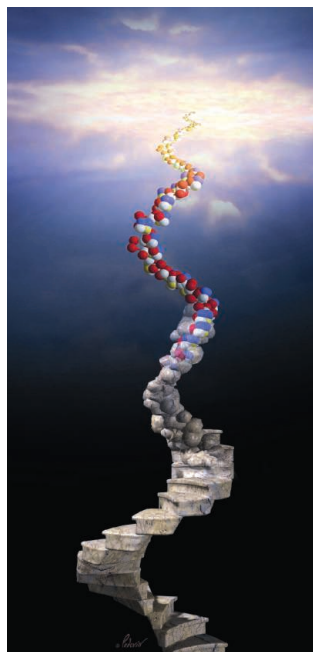
messenger RNA (1977). In 1971, polyadenylate [poly(A)] segments on active mRNAs were reported by three labs; poly(A) stretches of the same size were also detected in hnRNA. Next, methylated cap structures were identified both on mRNAs and on their suspected

precursors, the hnRNAs. But, how could much larger RNA molecules be converted to shorter ones and yet preserve both termini? Darnell recounts the time a post-doc came into his office one morning in 1975 and suggested, “Jim, maybe you cut out the middle and join both ends.” Darnell laughed him off. But only several years later did elegant studies of adenovirus mRNAs lead to the extraordinary conclusion—buttressed by observations from uninfected cells—that mammalian genes are in fact segmented into exons (which are expressed in the mRNA) and introns

(which are discarded from the hnRNA). Indeed, Darnell and the late Lennart Philipson had commented prophetically in 1971, “Detailed biochemical knowledge concerning the nuclear origin and processing of mRNA may therefore first become available from studies on [adeno] virus infected cells.”

Darnell’s book is not only a bible but a lexicon. Every key experiment from the past seven decades that shaped current appreciation of the multiple roles of cellular RNA molecules is described with clarity in several succinct sentences. The few figures are either directly reproduced from research articles or are carefully drawn models. The text is not cluttered with footnotes that beg for attention but invariably detract from the story line. The references are citations to original papers that established new concepts rather than to regurgitations by later review writers. If you know either the author or the subject, an experiment can easily be located through the index. The sense of history is always there. Often the references at the end of a sentence pair a citation from the 1980s with one from the 21st century. Thus, *RNA: Life’s Indispensable Molecule* should be required reading, as well as a handy desktop reference, for everyone charged with teaching gene expression on any level. It really does say it all.

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RNA

Life’s Indispensable Molecule

by James Darnell

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A DAY OUT: PALEOANTHROPOLOGY

Treasure Caves

Vanessa M. Hayes

All of humanity shares an African heritage. We are one, diverse species across the globe, with our roots in Africa. (1)

Genetic evidence suggests that modern human history began in southern Africa, a region also rich in ancient hominid diversity. South Africa's Cradle of Humankind World Heritage Site has made substantial contributions to the fossil record of these hominids.

First inscribed on UNESCO's World Heritage List in 1999, the Cradle of Humankind consists of 15 major fossil sites, encompassing hundreds of caves. The 470-km² core region, in the Witwatersrand basin about an hour's drive northwest of Johannesburg, includes the well-known sites Sterkfontein, Swartkrans, Kromdraai, and Malapa as well as the visitor center at Maropeng. Since its establishment, the Cradle of Humankind complex has been expanded to include two more distant components, each around 300 km from the core: the Taung skull site and the Makapan Valley.

My 9-year-old son Daniel and I began our visit at the Sterkfontein caves. For well over 3 million years, animals (including hominids) have fallen into the deep pits of the caves, becoming trapped and entombed. As we make our way underground, we are struck by the sharp change from the heat of the savanna to a constant 16°C. Our guide warns us of the dangers of snakes that still fall into the clutches of the caves; we note his emergency kit. He points out the ripple marks within the dolomite limestone walls (recording the shallow sea that covered the area 2.6 billion years ago) and the stromatolites (laminated mounded structures built up by ancient microorganisms). He explains how the caves began to develop some 20 million years ago. We are, however, here to walk with our ape-man ancestors, *Australopithecus*.

The first australopithecine remains (the skull of a 3-year-old) were discovered in 1924

in the North West Province of South Africa. Anatomist Raymond Dart described Taung child and named the species *Australopithecus africanus* (2). He noted the humanlike features of this 2.5-million-year-old (Ma) specimen, such as the small canine teeth, the lack of brow ridges, and, most importantly, the evidence that the primate was bipedal. Subsequent discoveries indicate that *A. africanus* roamed the then-forested region of southern Africa.

The almost 600 hominid fossils recovered from the Sterkfontein caves provide the most comprehensive glimpse into this extinct species. The two most famous *A. africanus* remains from Sterkfontein are Mrs. Ples (more likely "Mr."), an almost complete 2.05-Ma adult skull (discovered in 1947 by

Cradle of Humankind
World Heritage Site
Sterkfontein 1911, South Africa
www.cradleofhumankind.co.za



Rich diggings. The excavation site at Sterkfontein caves.

Robert Broom and John T. Robinson), and Little Foot. The first parts of the latter were collected (but not recognized) in 1980, and the remainder of the nearly complete skeleton was discovered by Ronald Clarke in 1997. As we descend into the dimly lit caves (not recommended for the claustrophobic), we walk past the iron gates and barbed wire that protect the part of Little Foot that remains in the caves. For over 13 years, researchers have been working to free the 2.2- to 3.3-Ma fossil from the limestone breccia. Our guide informs us that the hope is that the Little Foot excavation will be completed within a year. Having the entire specimen available for study will help resolve questions such as whether it is an early form of *A. africanus* or represents a previously unrecognized species of *Australopithecus*.

In the local Setswana language, Maropeng means "returning to the place of our origins."

At this location, between 500,000 and 1 million years ago, hominins used local rocks for making tools such as handaxes and cleav-

ers. Housed in the Tumulus building (evocative of an ancient burial mound), the Maropeng Visitor Centre contains exhibits on Earth history, the formation of fossils, evolution, and extinction, as well as replica casts of all the major hominins and lifelike illustrations (by the renowned paleoartist John Gurche). The displays are up to date: At the end of our interactive self-guided tour, we encounter the actual specimens of *A. sediba*, the most recent addition to the *Australopithecus* tree. A juvenile male and an adult female skeleton dated at nearly 2 Ma were discovered by Lee Berger and his team at the Malapa site and described

only last year (3, 4). My son is only a few inches taller than the age-matched boy, Karabo ("answer" in Setswana). The display introduces us to the scientific reports as well as the debate on how the southern African *Australopithecus* species might reshape our understanding of the hominin tree—by providing a controversial link between the oldest *Australopithecus*, 3.2-Ma Lucy (*A. afarensis*) from East Africa, and our genus *Homo*.

Visitors can easily spend a full day perusing the exhibits at Maropeng and Sterkfontein. The displays convey the excitement, increased understand-

ing, and still-unanswered questions raised through the discoveries of fossil hominids around the world. And they help us develop a picture of the complex patterns of hominid diversity over the past 7 million years. Clearly, Africa has been the central locus of hominid existence through time.

Our short visit to the Cradle of Humankind World Heritage Site reminded me of the substantial role Africa, and specifically southern Africa, has played in the mapping of our hominid history. I wonder how many secrets still lie hidden beneath the soils of this beautiful continent and within the genetic signatures of all modern humans.

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RESEARCH AGENDA

Guiding Limited Use of Chimpanzees in Research

Bruce M. Altevogt,^{1*†} Diana E. Pankevich,^{1*} Andrew M. Pope,^{1*} Jeffrey P. Kahn^{2*†}

Research on chimpanzees is contentious, expensive, and of increasingly limited necessity. Over the past 10 years, there have been only 110 projects involving chimpanzees sponsored by the National Institutes of Health. Close to half of these projects were used for hepatitis research; the remaining studies were in fields including comparative genomics, neuroscience and behavioral research, and infectious diseases such as respiratory syncytial virus (RSV). NIH requested that the Institute of Medicine (IOM) and National Research Council convene an expert committee to assess the current and future scientific necessity of chimpanzee use as a research model in publicly funded biomedical and behavioral research. The committee developed a set of uniform principles around what constitutes necessary use of chimpanzees, because of the lack of existing criteria (1).

- The knowledge gained must be necessary to advance the public's health;
- There must be no other research model by which the knowledge could be obtained, and the research cannot be ethically performed on human subjects; and
- The animals used in the proposed research must be maintained either in ethologically appropriate physical and social environments or in natural habitats.

Necessity of Chimpanzees for Research

Criteria specifically aimed at comparative genomics and behavioral research were as follows: (i) studies provide otherwise unobtainable insight into comparative genomics, normal and abnormal behavior, mental health, emotion, or cognition, and (ii) all experiments are performed on acquiescent animals, using techniques that are minimally invasive and in a manner that minimizes pain and distress. Acquiescence is

defined as situations in which animals do not refuse or resist research-related interventions and that do not require threats for participation; for instance, animals are often trained to present their arms for the purpose of blood draws. The committee found that much of the current comparative genomics and behavioral research met the criteria if minor modifications in study protocols were made to address questions of acquiescence, pain, and distress. Some studies did not specify the type of housing or how often and by what mode anesthesia was provided to the animal. In general, for behavioral research, the committee would not find it acceptable for an animal to be provided general anesthesia through a dart gun or for animals not to be housed in social settings. It encouraged studies relying on examinations or sample gathering performed as part of regular veterinary care.

Examples of minimally invasive research include behavioral observation and noninvasive interventions that can only be performed on anesthetized animals, e.g., magnetic resonance imaging scans of the brain. The committee recommended that these be performed in conjunction with regular veterinary exams and care that would otherwise require general anesthesia of individual animals. The committee's criteria for behavioral and genomics studies were additionally motivated by the view that research involving animals that are stressed or otherwise affected by their environment are less likely to yield meaningful, consistent, and useful results.

In addition to the principles described above, one additional criterion was specific to biomedical research: Forgoing the use of chimpanzees for the research in question will significantly slow or prevent important advancements to prevent, control, and/or treat life-threatening or debilitating conditions. The committee concluded that, although the chimpanzee has been a valuable animal model in the past, by its criteria, most current use of chimpanzees for biomedical research—including RSV vaccine, therapeutic hepatitis C virus (HCV) vaccine, and HCV antiviral drug development—is not

Chimpanzee research should not be banned, but its scientific necessity is very limited.

currently warranted, except for two limited research uses: for monoclonal antibodies and in prophylactic HCV vaccines.

Production of monoclonal antibodies after immunization in other species or through in vitro synthetic methods is equally powerful for the generation of such reagents. Further, there are currently four methods in use that lessen the need for safety tests in chimpanzee: (i) genetic engineering of the target protein in rodents, (ii) selection of antibodies that recognize target epitopes shared across species, (iii) selection of multiple antibodies that can serve as surrogates for responses, and (iv) testing low levels in humans (2). However, not all antibodies under development were derived with the newer technologies; studies based on these antibodies may require continued use of chimpanzees to avoid substantially slowing research or preventing important advancements. For these specific cases, continued use of chimpanzees should be assessed against the committee's criteria for biomedical research, although the expectation is that, within 5 years, chimpanzees will not be used in monoclonal antibody research.

The greatest number of projects using the chimpanzee over the past 10 years has been for hepatitis research. Development of antiviral drugs and therapeutic vaccines for HCV does not necessitate use of the chimpanzee because of the emergence of other animal models and the ability to ethically perform safety and efficacy studies in humans. However, the challenges associated with developing prophylactic HCV vaccines may continue to necessitate the chimpanzee.

Progress is being made in the development of various mouse models that can be infected with HCV, but these do not allow evaluation of the human protective immune response. Although challenge studies for HCV prophylactic vaccine development cannot be performed in humans, studies in consenting individuals at high risk for natural HCV infection can be ethically done provided that these vaccines are first shown to be safe and immunogenic in experimental animals, such as mice and nonhuman primates. The committee could not reach consensus on whether preclinical studies exam-

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*Responsibility for the content of this article rests with the authors and does not necessarily represent the views of the Institute of Medicine, its committees, and its convening activities. †J. P. Kahn was chair of the IOM committee (1).

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ining the safety of candidate prophylactic vaccines necessitate the chimpanzee, or if the development of a prophylactic HCV vaccine would be slowed by bypassing preclinical chimpanzee research and going directly into human trials. Research relying on existing blood or tissue samples would be exempt from the criteria, as no interaction with living chimpanzees would be required.

Finally, the committee stressed that chimpanzees used for any research must be maintained in ethologically appropriate environments accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, for example, Primadomes or corrals with environmental enrichment, outdoor caging with access to shelter, and indoor caging (1).

Discussion

The committee was charged specifically to develop criteria for chimpanzee use; therefore, it would not be appropriate to apply these criteria to assess the necessity of other nonhuman primates in research. The necessity of other research animals should continue to be assessed using current regulations and guidelines.

The Interagency Animal Model Committee (IAMC) currently provides an additional review by the NIH to ensure the appropriateness of using the chimpanzee and the number of animals, as well as the degree of invasiveness of the research. However, only federal employees are members. The assessment would be strengthened and the process made more credible by establishing an independent oversight committee that builds on the IAMC and that uses the recommended criteria. It should include public representatives, as well as individuals with scientific expertise in the use of chimpanzees and alternative models and in areas of research in which chimpanzees might be used. This is similar to the working groups of the Advisory Committee to the NIH Director.

The committee was not tasked with assessing the use of the chimpanzee by the private sector; however, many of the biomedical research areas assessed by the committee are also being pursued by industry. Adoption of the committee's criteria by industry could ensure that the private sector limits its use of chimpanzees to areas of clear necessity.

The request for the IOM report originated with the U.S. Congress, which for the past three sessions has been considering legislation that would ban research using the chimpanzee and other great apes [Great Ape Protection Act (GAPA) of 2010 (111th Congress, second session) and Great Ape Pro-

tection and Cost Savings Act (GAPCSA) of 2011 (112th Congress, first session)]. Similarly, in 2010, the European Union banned the use of all great apes, including chimpanzees, in research. However, a safeguard clause in the EU "ban" allows use of great apes for research that is broadly defined as addressing a life-threatening, debilitating condition that endangers human beings "pro-

... it will be very difficult to defend the necessity of nearly all current biomedical research on chimpanzees.

vided the purpose cannot be achieved by the use of other species or alternative methods" (3). The EU Directive provides no additional information by which researchers or member states should base their decisions. There has been no biomedical research involving great apes in the EU since 1999 (4, 5).

Both the committee's criteria and the EU directive are in contrast to the approach proposed in GAPCSA, which would prohibit "any research that may cause death, injury, pain, distress, fear, or trauma" (H.R.1513, IH and S.810.IS). The proposed legislation does not include an analysis in support of the proposed prohibition, though its "findings and purpose" focus on the welfare and protection of the animals. The committee did consider ethics in its analysis, which informed its approach in responding to the NIH charge to assess the necessity of the chimpanzee in contemporary and future research (1).

Given the information available to the committee through its research and provided by relevant federal agencies, it will be very difficult to defend the necessity of nearly all current biomedical research on chimpanzees. The most difficult assessment will be in cases of prophylactic vaccine development research in which the chimpanzee is the only nonhuman animal model. Even those cases are likely to see the need for chimpanzees diminish as alternative models become available. However, a new, emerging, or reemerging disease or disorder may present challenges to treatment, prevention, and/or control that defy non-chimpanzee models and technologies and thus may require their future use. Therefore, an outright ban on biomedical chimpanzee research would not be appropriate.

Since 1997 the NIH has instituted a moratorium on government-sponsored breeding of chimpanzees (6); however, very limited

breeding of chimpanzees for research purposes is still under way through the support of the private sector. The moratorium presents a challenge for conducting future research, should it be justified, because of the aging chimpanzee population (7), the very small number of research-naïve animals, and the time it takes for chimpanzees to mature. The cost of maintaining a chim-

panzee is \$44/day, and with an average life span of 40 to 45 years, this can reach over \$642,400 per animal over the course of their lifetimes (there has been a moratorium on euthanasia of chimpanzees since 1995) (8). Should there be a public health emergency requiring the chimpanzee, it will likely not be possible to quickly breed and expand the population. Therefore, the number and demographics of animals maintained for any potential research use will need to be carefully considered.

The committee's review and assessment highlighted not only the issues and challenges of chimpanzee research but also the importance of other animal research models. Although the necessity of chimpanzees in research is quickly declining, animal research remains a critical tool in protecting and advancing the public's health.

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BEHAVIOR

How Honeybees Break a Decision-Making Deadlock

Jeremy E. Niven

For a honeybee swarm of potentially thousands of individuals, choosing a home is a momentous decision. Failing to choose a single location may cause the swarm to split and the queen to be lost (1); choosing poorly may limit the swarm's growth or expose it to freezing temperatures during the winter (2). Studies over the past 60 years have shown that honeybee swarms use quorum sensing, a form of decentralized decision-making, to choose a suitable nest site, but many gaps remain in our understanding of this process. On page 108 in this issue, Seeley *et al.* (3) show that an inhibitory signal between bees advocating different locations allows them to make a decision even when potential nest sites are equally favorable.

Honeybee colonies reproduce through budding, whereby the queen and some workers leave the nest and bivouac on a branch. Some of the most experienced workers leave to locate suitable nest sites (4). Upon their return, these scouts advertise potential locations and their qualities by performing a waggle dance. During the dance, the scout walks straight across the bivouacking bees, making side-to-side waggles of her body. She then stops, turns left or right, and walks a semicircular return path to her starting point. The waggle run's duration and orientation encode the length and the angle of the outward flight, respectively, whereas the number of dance circuits

encodes the quality of the potential nest site (5). Waggle dances recruit additional scouts to a site until a quorum number is reached and the swarm prepares to move to its new home (2).

Scouts advocating less attractive sites produce fewer dance circuits and make fewer trips to the site (6). Along with the recruitment of uncommitted scouts to more attractive sites, this was assumed to be sufficient to enable the bees to reach a quorum, thereby deciding which site to choose (2). However, foraging workers use an additional type of signal to communicate with other bees. Upon returning from a feeder that is crowded or where a predator is present, forager bees produce a brief vibrational signal that discourages other bees from producing waggle dances that advertise the location of that feeder (7). Hypothesizing that a similar signal may be used by house-hunting bees, Seeley *et al.* set out to observe scout behavior. They found that scouts received "stop" signals—head butts mainly to their head and thorax—from other bees during the return run of the waggle dance (see the figure). These stop signals occurred more frequently just before a scout stopped dancing.

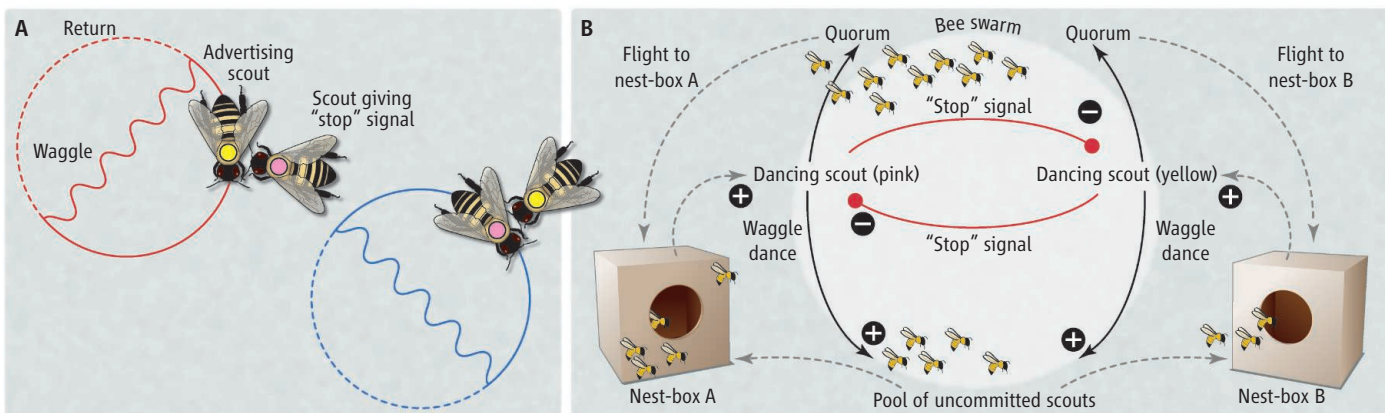
The authors next established swarms on Appledore Island (Maine), which lacks natural nest sites, and gave them a choice of two identical nest boxes. Scouts visiting one box were marked with yellow paint; those visiting the other were marked with pink paint. Most of the bees giving "stop" signals had paint marks, showing they were scouts. During the decision phase of the nest-site

During the search for a new nest site, use of an inhibitory signal enables honeybees to reach a decision.

selection process, dancing scouts with yellow paint received many more stop signals from scouts with pink paint and vice versa, showing that scouts from one site preferentially inhibit the dances of those advertising a competing site (see the figure, panel A). Once the scouts started implementing the decision, dancing scouts received stop signals from scouts that had visited either site. When swarms were given only one nest box, scouts received few stop signals during the decision phase but many during the implementation phase. This general inhibition of dancing during the implementation phase presumably ensures that all the bees are present when the swarm takes flight.

To demonstrate a role for the observed cross inhibition between scouts advertising competing sites, Seeley *et al.* constructed a series of computational models of the collective decision-making process, based on the interaction rules they had observed among the scouts. Models that incorporated no or indiscriminate stop signaling predicted that the scouts would reach a stable deadlock, failing to choose between two

Cease and desist. (A) Seeley *et al.* have found that during house hunting, scouts advertising one nest site preferentially inhibit scouts advertising another site during the decision-making process. Inhibition is conveyed by a "stop" signal, given mainly to the head and thorax of a scout during the return phase of the waggle dance. (B) Stop signals from scout bees inhibit other scouts, discouraging them from advertising a potential site. These inhibitory stop signals combine with recruitment of neutral scouts to produce a winning site.



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equally suitable nest sites. Only a model incorporating sufficiently high levels of cross inhibition predicted that the scouts would converge rapidly upon a decision.

The authors compare the interactions among scouts with those among neurons in primate decision-making networks, which can be modeled with similar interactions (8). They argue that populations of bees and neurons can be considered to be mutually inhibitory, leaky integrators of sensory evidence that lead to a choice when they exceed a threshold (see the figure, panel B). These features are not restricted to neural circuits but underpin decisions in many biological processes, such as pattern formation (9, 10).

Although Seeley *et al.* gave the bees a choice between two identical nest sites, house-hunting swarms typically evaluate many sites of differing quality. This may require scouts to distinguish waggle dances advertising several other sites from their own, possibly through forming categories of “same” and “different,” or more likely through odor cues. In this situation, scouts may produce stop signals in proportion to the quality of the site they have located,

which would produce effects similar to lateral inhibition in neural circuits and cell fate decisions (9–12). By averaging surrounding activity, lateral inhibition can reduce the effects of intrinsic noise and maintain sensitivity over a wide range of stimulus intensities (11, 12). Thus, as well as reinforcing asymmetries vital for resolving deadlocks, inhibitory signals of scouts may be important for reducing noise at the beginning of the decision-making process and for rescaling the process when there are many good alternatives.

The findings of Seeley *et al.* emphasize the computational value of signals involved in decision-making in house-hunting honeybees. The stop signal has either been co-opted to or from foraging, but in doing so its use has switched; scouts use the stop signal to inhibit bees advertising different sites rather than the same site, and they do so because of the high, rather than low, quality of the site they have visited. Thus, inhibitory (vibratory head butt) and excitatory (waggle run) signals can be used to form behavioral circuits (foraging, house-hunting), just as they are in the nervous sys-

tem to form neural circuits. Future research stands to benefit from this computational approach, incorporating behavioral analysis with concepts and ideas from neuroscience and developmental biology.

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GENETICS

Variable Outcome of Mutations

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Why do some mutations produce a particular trait or disease, whereas others only increase the likelihood of such an outcome? One explanation is that other variations in the DNA sequence strengthen or weaken the effect of a given mutation, although environmental factors may also have an impact. On page 82 of this issue, Casanueva *et al.* (1) show that in the nematode *Caenorhabditis elegans*, stochastic differences in the amount of a protective chaperone protein, after exposure of the worms to mild stress, influence the effect of mutations.

In some cases, the link between an individual's genotype (DNA code) and phenotype (traits, diseases) is straightforward. For example, individuals with more than

41 cytosine-adenine-guanine repeats in the Huntingtin gene will invariably develop Huntington's disease. However, many variations in the DNA sequence do not always produce a certain trait or illness. Women carrying the *BRCA1* mutation have about a 65% chance of developing breast or ovarian cancer before the age of 70 (2). Why don't all women carrying the mutant allele develop these cancers? One reason is that other genomic variation may influence the effect of a given mutation (3). For example, certain alleles of the *TNRC9* gene increase the effect of having the mutant *BRCA1* allele (2, 4). However, when a large number of loci show genetic interactions, it becomes difficult to predict the importance of a given variation (5), much less an individual outcome.

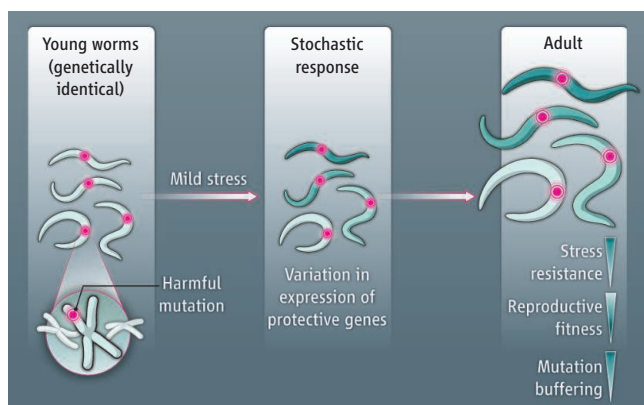
Environmental factors may also influence the effect of a mutation (3), but their functional consequences can be difficult to unravel. Casanueva *et al.* show that *C. elegans* worms with a potentially deadly mutation in the transcription factor–encoding

Environmental stress and stochastic differences in the expression of protective genes influence the effect of mutations.

gene *lin-29* have a 35% better chance of surviving if they receive a mild heat stress at an early developmental stage (see the figure). The authors reason that heat stress induces the expression of molecular chaperones that protect cells by preventing misfolding and aggregation of other proteins. However, chaperones can also protect against harmful effects of some mutations, possibly by reducing the chance that a mutation leads to protein misfolding (6). Indeed, worms exhibiting a stronger stress response (as measured by gene expression) to heat exposure had reduced mortality, and those with a constitutively activated stress response also showed better survival, even in the absence of any stress stimuli.

Individuals not only differ in their DNA code and their environment, but also exhibit seemingly random (stochastic) differences in the transcription of some genes (7, 8). Casanueva *et al.* found that stochastic differences in the expression of protective chaperone genes (in the absence of a stress treatment) also reduced the effect of mutations; indi-

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The final effect. A mild stress response protects genetically identical young worms against deleterious mutations. However, because of individual variation in stress signaling, the extent of this protection varies. When such worms carry a potentially harmful mutation, individuals with high stochastic expression of certain stress-protective genes (dark green) show better survival than individuals with lower expression (light green), but at a cost of reduced fecundity. Although genetically identical, the starting population already has variable expression of stress-protective genes, which also affects the outcome of mutations (not shown).

chance of surviving in both benign and stress-inducing conditions than a population exhibiting uniform gene expression.

Although genetic individuality is emerg-

ing as a central theme of research, underlying promising initiatives in personalized medicine, Casanueva *et al.* caution that “nongenetic individuality” (12) extends beyond unicellular organisms and has important functional consequences. Even though a person’s genome sequence may be available, with all genetic interactions completely unraveled, and life history fully known, other, non-genetic differences may constitute an elusive determinant of risk for disease. It is unclear whether the findings of Casanueva *et al.* can be simply extrapolated to humans, especially because such a risk diversification strategy may prove particularly useful for self-fertilizing organisms such as *C. elegans*. Moreover, only temperature-sensitive mutations were affected by differences in chaperone expression, suggesting that this phenomenon may be

restricted to a subset of mutations. Nevertheless, given the conservation of stress response mechanisms and chaperones from yeast to humans, we may have to consider that destiny is not wholly determined by DNA, and that sheer luck—in the form of stochastic variation in gene expression—also has a say.

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viduals with greater chaperone expression were more resistant to deleterious mutations. Even early worm embryos differ in their chaperone expression, which may influence the outcome of mutations in each individual (9). Moreover, higher stochastic chaperone levels are correlated with extended life span (10), and the inverse also holds true: A mutation can increase the stochastic variation in the expression of certain genes, which can in turn determine mutation outcome (11).

Given the positive effects of the chaperones, why don’t all worms exhibit high chaperone expression levels? Casanueva *et al.* found that individuals with high chaperone expression produced fewer offspring (reduced fecundity). Stochastic variation in chaperone expression may therefore represent a bet-hedging strategy, allowing populations with varying chaperone expression to have a better

APPLIED PHYSICS

Ohm’s Law in a Quantum World

David K. Ferry

Ohm’s law is an empirical law based on the observation that the electrical transport properties of materials exhibit linear behavior. The principle is that the voltage that develops across a piece of conducting material is linearly proportional to the current flowing through it; the constant of proportionality being termed the resistance, $V = IR$ (1). Such behavior is the backbone of classical conduction in these materials. In the 1920s and 1930s, it was expected that classical behavior would operate at macroscopic scales but would break down at the micro-

scopic scale, where it would be replaced by the new quantum mechanics. The pointlike electron motion of the classical world would be replaced by the spread out quantum waves. These quantum waves would lead to very different behavior. On page 64 of this issue, Weber *et al.* (2) have constructed atomic-scale nanowires in Si and have observed that Ohm’s law remains valid, even at very low temperatures, a surprising result that reveals classical behavior in the quantum regime.

Transport in the quantum world is characterized by coherence, where the phase behavior of the waves remains undisturbed over sizable distances (typically on the scale of the sample size). This coherence leads to wave interference, such as the Aharonov-Bohm

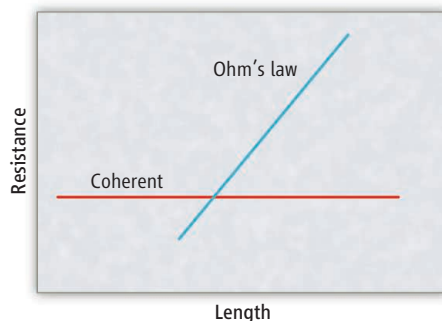
Conductivity measurements reveal unexpected classical electronic behavior at the quantum level.

effect in conducting rings (3), where waves traveling around different paths (an impurity or defect, for example) can interfere with each other. Coherent transport in nanowires is determined by the number of transverse wave modes that propagate in the constrained geometry (4, 5) (analogous to the different waves on a plucked string). In this regime, the resistance of the nanowire is given by the Landauer formula (6), $R = h/(2e^2N)$, where N is the number of the modes, e is the electronic charge, and h is Planck’s constant. Note that the conductance, and hence the resistance, is independent of the length of the sample of material being investigated (see the figure, red curve). In the classical world, the resistance depends linearly upon the length—a

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longer piece of material requires more voltage and hence has higher resistance (blue curve). Here, the resistance is given by $R = L/\sigma A$, where L is the length of the sample, A is the cross-sectional area, and σ is the conductivity. Weber *et al.* observe linear dependence of the resistance upon the length down to ~ 10 nm, which implies that classical behavior was maintained, even at low temperature. What breaks up the coherence, and leads to the classical behavior, is dissipation arising from collisional processes in the material, and this is expressed within the conductivity.

In a given material, variations in temperature or in electron scattering can move the blue curve up or down. The resistance from various mechanisms are additive, so that the total resistance can follow the quantum curve until it intersects with the Ohm's law curve and will then follow the latter. Hence, there is a crossover region where the two curves meet. In materials that are more conducting than silicon, such as nanowires in indium arsenide, experiments (7) have shown that this crossover occurs for lengths of almost 200 nm, even at room temperature, and this has been confirmed by calculations (8). It is clear that the onset of Ohm's law depends on the strength of the scattering of the electrons within the nanowire. In the work of Weber *et al.*, the number of phosphorus atoms, and hence the number of electrons, is on



Ideal behavior. In systems where quantum coherence governs the transport, the resistance is independent of the length of the sample of material (red curve). Classical Ohm's law requires that the resistance be linearly proportional to the length (blue curve). Hence, for a given sample, a crossover from quantum to classical behavior where the two curves meet is expected. The measured resistance would be a sum of these two curves. Weber *et al.* (2) report classical behavior under conditions where quantum effects would be expected.

the order of 10^{21} cm^{-3} . This very high doping means that the scattering, and the resistance are much larger, which will lead to a shorter coherence length, and they found that this crossover had to be below 10 nm even at their low temperature.

The implications of having Ohm's law satisfied at such low temperatures are interesting. Normally, we would expect to study pure quantum effects at low temperatures,

but the results here suggest that more care will be needed to sort quantum effects from classical ones. This is also not good for concepts that use phosphorus atoms as qubits in quantum computing (9, 10), because the coherence needed becomes questionable. On the other hand, this may well pave "the way for ultra-scaled classical as well as quantum electronic components..." (2). This is good news for the semiconductor industry, which seeks to extend Moore's law [see, e.g., (11)] for several more years, a process enabled by scaling individual devices to smaller sizes. It has been thought that quantum effects would limit this in the near future, but the results presented by Weber *et al.* suggest that several generations are still possible.

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PLANT SCIENCE

Controlling Hormone Action by Subversion and Deception

Jeffrey Leung

The Nobel chemist Roald Hoffmann described molecular mimicry as a mechanism founded on subversion and deception. On page 85 of this issue, Soon *et al.* (1) report intriguing evidence for such mimicry in the transmission of signals elicited by the plant hormone abscisic acid (ABA). Using the model plant *Arabidopsis thaliana*, the authors show that the ABA-bound receptor and kinase alternately bind to a phosphatase, turning on and off this stress-responsive pathway, respectively. Not surprisingly, ABA is behind this

"subversive" dealing in partner swapping.

ABA is a terpenoid that promotes seed maturation, prevents germination, and curtails excessive water loss during vegetative growth. In the face of climate warming and water shortages, modifying ABA synthesis or perception as possible solutions to sustain crop yield for food and fuel has attracted the interests of policy planners and scientists.

The core ABA signaling module involves three different types of proteins (2): the PYR/PYL/RCAR family of ABA receptors; clade A type 2C protein phosphatases (PP2Cs, including ABI1, ABI2, and HAB1); and group III SUCROSE NONFERMENTING1 RELATED kinases (SnRK2.2, 2.3, and 2.6). These three protein types are necessary and sufficient to mediate an ABA-

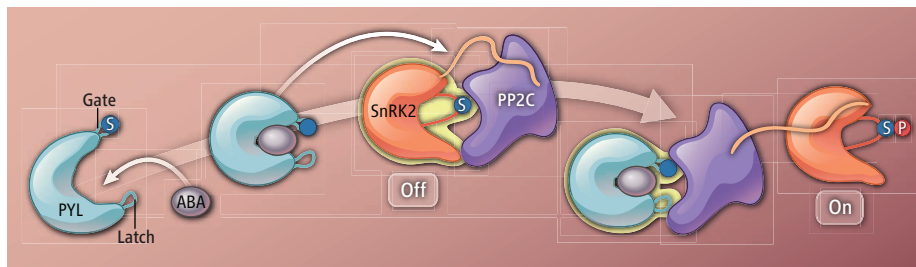
Molecular mimicry between a plant hormone receptor and a kinase allows them to swap binding to a phosphatase to control a signaling pathway.

triggered model signaling cascade in vitro (3).

ABA is cradled deep inside the receptor and is enclosed by conformational changes of the two substructures called "gate" and "latch" (4). The "gate" creates a new surface on the receptor that can tether the PP2C, freeing the SnRK2 to autophosphorylate (Ser¹⁷⁵ in the activation loop of SnRK2.6) and subsequently phosphorylate downstream targets. In the absence of the hormone-receptor complex, the PP2C turns off the signaling pathway by dephosphorylating the SnRK2. Although ABI1, ABI2, and HAB1 interact interchangeably with SnRK2.2, SnRK2.3, and SnRK2.6 (5–10), the functional importance had not been clear.

Soon *et al.* show that besides the catalytic removal of the phosphate from the phospho-

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Partner swap. Molecular mimicry between the kinase SnRK2 and the hormone receptor PYL bound to ligand ABA permits alternate binding to the PP2C phosphatase. This change in partners activates (on) or deactivates (off) SnRK2, allowing it to phosphorylate downstream signals.

serine in the activation loop of SnRK2.6, the binding further suppresses any remaining kinase activity. To uncloak the details of this two-step mechanism, a fusion protein HAB1-SnRK2.6 was generated as a linear peptide. By incorporating entropy-decreasing mutations, the authors coaxed the fused protein into high-quality crystals. Structural analysis of the complex revealed that the kinase SnRK2.6 mimics the face of the ABA receptor that binds to the phosphatase HAB1. The major contact surface between this kinase and phosphatase runs along their catalytic sites. Like the “gate” structure of the ABA receptor in the ABA-PYL-PP2C complex, the activation loop of the kinase SnRK2.6 also inserts into the catalytic center of the phosphatase HAB1. The cleft between the “gate” and “latch” of the receptor is also mimicked by a pocket in the C-lobe region of SnRK2.6. The contact between the α G helix of SnRK2.6 and a loop structure in HAB1 is adjacent to the HAB1 Trp³⁸⁵-PYL interaction loop. Thus, the receptor, once bound by ABA, adopts a surface structure remarkably similar to that of the kinase (see the figure).

ABA-PYL-PP2C and the SnRK2-PP2C complexes are not mutually exclusive as expected. ABA-PYL-SnRK2-PP2C in fact forms a quaternary complex. This is because, in addition to the kinase catalytic domain, a second acidic motif of ~25 residues called the ABA box found in the C terminus of SnRK2.2, SnRK2.3, and SnRK2.6 can also interact with HAB1 (but not with the receptors). The other seven SnRK2 members in the family have no obvious ABA box, but the overall amino acid compositions in the coincidental regions are acidic. With the exception of two SnRK2 enzymes, each of the other five acidic motifs also interacts with a subset of the PP2Cs. HAB1 can bridge the interaction between the receptor PYL2 and SnRK2.6 in the presence of ABA, but not in its absence. This means that the ABA-bound receptor can dislodge the kinase from the phosphatase without fully dissociating these two proteins;

the kinase will remain tethered by its ABA box to the phosphatase. This also explains why the PYL2-HAB1 complex can be disrupted by using increasing concentrations of the isolated kinase domain of SnRK2.6, whereas the ABA-bound receptors cannot dissociate SnRK2-PP2C, even if the ABA-bound receptors have much stronger affinity for the phosphatases relative to the kinases.

Soon *et al.* hint that molecular mimicry might be a common mechanism in many biological processes involving kinase-phosphatase complexes. Tantalizing parallels can be found in the catalytically inactive pseudophosphatases EGG-4 and EGG-5 in mammals, which recognize the activa-

tion loop of the kinase MBK-2 and block its kinase activity. The mammalian AMPK-PP2C α complex (homologs of the SnRKs and PP2Cs of *Arabidopsis*) might also pack their catalytic sites to maintain the kinase inactive. The structural studies on the core ABA signaling proteins establish a new paradigm for kinase-phosphatase co-regulation and coevolution. Indeed, subversion and deception by competitive binding of these major cell signaling proteins in biasing important biological decisions may turn out to be pervasive.

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APPLIED PHYSICS

Plasmonic Modes Revealed

Philip E. Batson

Time-resolved electron microscopy can map the electric field created in and around a nanoparticle by photonic excitation of its plasmonic modes.

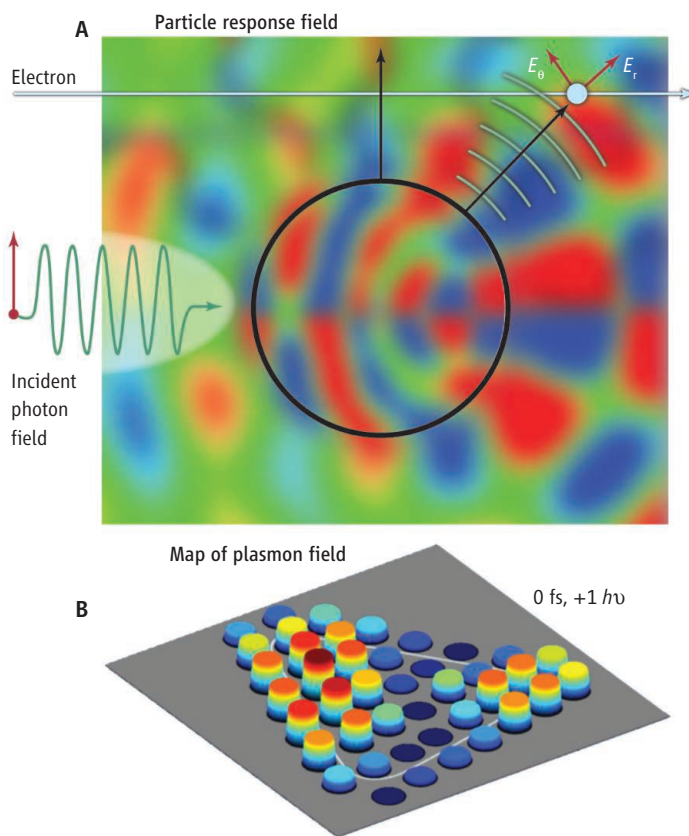
When nanostructures made of metals such as gold and silver are illuminated with visible light, plasmonic modes can be excited that cause conduction electrons to oscillate. This motion creates a pattern of electric fields, extending both within and outside the structure, that can be tuned by changing the particle size and shape to efficiently couple light to electronic processes. Practical applications of this coupling include improved harvesting of light for photovoltaics (1) and enhanced sensitivity for sensors based on light-emitting messenger molecules (2). Although there is well-developed theoretical understanding of how photons interact with nanostructures that are

much smaller than their wavelength, we have few methods for measuring electric fields nearby and within nanoscale structures during photonic excitation. On page 59 of this issue, Yurtsever *et al.* (3) report using time-resolved electron energy gain/loss spectroscopy in an electron microscope to obtain spatially resolved maps of electric fields that result when nanoscale metal objects are illuminated by incident photons.

Previously, optically induced plasmonic electric fields have been probed by near-field scanning optical microscopy (NSOM) and its variants that use metal tips (4, 5). In NSOM, a subwavelength optical aperture samples photon intensity as a function of position. However, spatial resolution and collection efficiency are both limited because NSOM samples only the decaying or evanescent part of the optical signal outside the nanoscale object.

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Field day for plasmons. (A) The theoretical basis for the experiment of Yurtsever *et al.* is shown. A kiloelectron-volt electron interacts with the evanescent electric field created by a photon exciting a metal nanocylinder (shown in cross section). Blue and red colors indicate the phase of the plasmon response field E_θ and E_r ; near-zero responses are in green. Depending on the arrival time of the electron, it can gain or lose energy by interacting with this field. [Adapted from (14)] (B) A map of the intensity of electron energy gain as a function of position on a triangular nanoscale silver particle. The map integrates scattering through or past the nanoparticle, along the electron trajectory.



Electron energy-loss scattering can also efficiently create surface plasmon modes within groups of nanoscale metal objects, allowing spatial investigation of resonant plasmonic response. This technique supported understanding of surface-enhanced Raman scattering on silver surfaces with nanoscale roughness, and suggested interesting possibilities for engineering optical coupling to nanoscale systems (6, 7). The possibility of deliberate acceleration of electrons by light, mediated by gratings and surface-guided modes to match the momentum transfer required by electrons (8, 9), has also been considered. Finally, electron energy-gain spectroscopy might combine nanoscale spatial resolution with the energy selectivity of photons (10).

Observation of electron energy gain has had little success, however, apparently because of the difficulty of coupling enough light into the nanoscale object so that each incident electron would have sufficiently high probability for interaction with plasmonic fields. Electron-beam currents in electron microscopy are so small that only about one electron per nanosecond interacts with the specimen, whereas plasmon decay happens on the femtosecond time scale, producing a timing mismatch of five orders of magnitude. Thus, electron energy-loss events that create a plasmon and scatter the electron simultaneously are quite common, whereas energy-gain events, which require absorption of a separately created plasmon, have not been observed previously.

During the past few years, Zewail's group (11) and others (12, 13) have introduced laser-excited photocathodes to electron microscopy, enabling time-resolved experiments. It

is now possible to optically excite or “pump” the specimen and then probe it with an electron at a later time. Yurtsever *et al.* convincingly demonstrate a direct interaction of high-energy electrons with surface plasmon fields excited by incident photons in single nanostructures. A theoretical model developed by Park *et al.* summarizes the experiment (14) (see the figure, panel A). In this case, the nanostructure is a metal cylinder (depicted as a circle, with its axis perpendicular to the page). A photon field incident from the left excites surface plasmons, producing response fields both inside and outside the cylinder. These fields are summarized by the background image, with red or blue colors denoting positive or negative phase of the response field. A high-energy electron is incident from the left, and, depending on the particular timing of its arrival, can be either accelerated or decelerated by the fields, providing energy gain or loss. In this illustration, the electron passes outside the cylinder, sampling evanescent fields beyond the cylinder surface.

In a semiclassical treatment, the electron is represented by a point charge, and the total energy gain or loss is determined by an integral over its path (15). The timing accuracy of the electron's arrival is limited to a few hundred femtoseconds by the laser and pulse width, as well as by the instrumental details of

the electron photogeneration and acceleration. Thus, electrons arrive during a spread of times around the arrival of the photon field, producing energy gain or loss, depending on the details of the surface plasmon response phase during the electron passage. If the response field is very strong, multiple quanta of energy can be transferred to or from the electron. In the case of a single triangular silver nanoparticle, many electrons undergo no loss, but some gain or lose one or two quanta of energy. The energy gain and loss peaks are exceptionally strong—several times the electron energy-loss scattering probability in the absence of the incident photon.

With such a strong set of energy gain and loss peaks, Yurtsever *et al.* produced maps of field intensity inside and outside nanoscale objects. An example for the energy gain of $+h\nu$ at a time delay of 0 fs from the triangular silver nanoparticle is shown in panel B of the figure. In scattering near the surface of copper, peaks situated at many discrete energy quanta were also evident. The experimental visualization of the optical response of a nanoscale dielectric object will be important for many applications, including renewable energy sources, continued scaling of semiconductor computation capabilities, and understanding of dielectric structures that exhibit unusual properties.

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RETROSPECTIVE

Lloyd J. Old (1933–2011)

Padmanee Sharma^{1,2} and James P. Allison^{2,3,4}

In 1958, Lloyd Old drove into New York in a red Corvette, which was a gift from his parents for having graduated at the top of his medical school class. He had completed his medical degree at the University of California, San Francisco, and made a crucial decision to pursue a research fellowship at the Sloan-Kettering Institute rather than further medical training. He was optimistic about science and especially tumor immunology, a field that was in its infancy. For the next 50 years, Lloyd grew this field into one with substantial evidence, helping to propel it into the realm of standard treatment for cancer patients. On 28 November, he died in his home in New York from prostate cancer.

Lloyd was born in San Francisco in 1933, and his career in biology began at the University of California, Berkeley. Once in New York, he remained at Sloan-Kettering to do his life's work. In the 1960s, he and Edward Boyse and Elisabeth Stockert initiated a comprehensive analysis of normal and malignant mouse lymphoid cells, which led to the identification of a range of cell surface antigens, including what is now called CD8 and the thymus leukemia antigen (now recognized as a nonclassical major histocompatibility complex molecule). These initial discoveries fueled his desire to understand how the immune system could reject tumors. During this time, his technicians, Elizabeth Carswell and Barbara Williamson, noted that tumors of mice turned black, which eventually led to the identification of the cytokine, tumor necrosis factor (which is involved in tumor regression). Lloyd graciously acknowledged Carswell and Williamson by making them first and senior authors on the 1975 paper that reported the data. Lloyd's generous nature was always present as he guided the laboratory through numerous scientific discoveries, including the use of attenuated Bacille Calmette-Guérin virus as tumor immunotherapy, the association of the tumor's suppressor protein p53 with cancer, and the use of serological methods to identify tumor antigens.



Lloyd was determined to apply the principles that he had learned by studying murine tumors to human cancers. This required establishing human tumor cell lines, which was performed in his lab for many years. The lab's rich source of melanoma cell lines led to the analysis of patients' T cells for reactivity against autologous tumor cells in the 1980s. Lloyd and his colleagues observed that patients with a specific T cell response to a particular antigen could have a favorable response to their disease. This led to the search for tumor antigens that could be used as vaccines to boost antitumor immune responses. However, early clinical trials of vaccines based on peptide antigen did not produce sufficient clinical outcomes, which generated skepticism about the therapeutic potential of tumor immunology. Lloyd was a visionary and understood the need for more rigorous studies in humans. He used to say that the clinic should be considered a laboratory where one could conduct experiments to understand immune responses in cancer patients. This was a difficult concept for many basic scientists and clinicians to embrace, but Lloyd persisted.

Lloyd realized that the field of tumor immunology required not only scientific effort, but also clear leadership. To facilitate small clinical trials with vaccines and novel immunomodulatory agents in cancer patients, Lloyd envisioned a new infrastructure that would require a team of translational investigators. He used his resources as Director and Chief Executive Officer of the Ludwig Institute for Cancer Research (LICR) and his position as a Founding Member and Scientific Director of the Cancer Research Institute (CRI) to establish, in 2001, a team under the umbrella of a new organization known as the Cancer Vaccine Collaborative (CVC). The LICR and the

An immunologist devoted his life's work to understanding how the immune system fights cancer.

CRI worked through the CVC to produce clinical-grade reagents for small immunotherapy trials, obtain regulatory approval for the trials, sponsor the trials, fund the trials at various academic institutions, and obtain patients' samples for immune monitoring. His mantra was "to vaccinate, you need to know how to immunize, and to immunize, you need to know how to monitor." Because Lloyd's lab had established methods for monitoring immune responses to a cancer testis antigen (NY-ESO-1), the clinical trials focused on this antigen, which tested specific concepts such as immunogenicity of a protein vaccine versus DNA vaccine, and immune responses elicited in the presence of different adjuvants or immunostimulatory agents. Lloyd planned for trial data to be integrated to provide a comprehensive picture of human immune responses against cancer and in the setting of different immunomodulatory agents.

Lloyd oversaw investigators at the CRI and took great pleasure in hosting its annual meetings as well as the symposiums organized by the CVC. He engaged with nearly everyone at these meetings and made it a priority to introduce participants to each other. He fostered a family atmosphere and was the doting patriarch. He answered countless telephone calls related to scientific questions or personal issues such as helping someone to get a doctor's appointment for a friend with cancer. His loyalty to science and the people in his life was unwavering. When Elisabeth Stockert became sick with pancreatic cancer, Lloyd brought meals to her in the hospital, coaxed her to eat, and talked with her for hours about the "good old days in the lab."

Lloyd was for many of us a friend, mentor, father figure, and soulful spirit filled with passion for life and learning. His first passion, the violin, taught him that great symphonies required many accomplished musicians to learn the notes so that they can be played together to achieve perfection. Lloyd allowed the many notes in tumor immunology to be played beautifully together by accomplished scientists so that his vision of harnessing the power of the immune system to eradicate cancer could be fulfilled. We consider it a gift to have known him and we will miss his gentle presence and insightful wisdom every day.

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PrP Antibodies Do Not Trigger Mouse Hippocampal Neuron Apoptosis

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According to the protein-only hypothesis, prions are composed of polymerized conformational isomers of host-encoded cellular prion protein (PrP^C) (1). Targeting PrP^C in mice with established brain infection blocks development of clinical disease and reverses early pathology and behavioral abnormalities (2, 3). Therapeutic proof of concept is provided by passive immunotherapy via intraperitoneal injection with ICSM18 or 35, monoclonal antibodies against PrP (4). However, a study that aimed to determine whether PrP^C-dependent signaling could produce neurotoxic effects in vivo suggests that hippocampal neurons degenerate after intracerebral injection with particular monoclonal antibodies against PrP, although this was dose- and epitope-dependent (5). Two of the three studied antibodies, immunoglobulin G (IgG) P and IgG D13, which bind to epitopes within the 95-to-105 region of PrP, cause extensive neuronal loss throughout the hippocampal region in the majority of C57BL/10 mice 24 hours after stereotaxic injection of 2-μl aliquots at a concentration of 1 mg/ml, whereas IgG D18, recognizing residues 133 to 157, did not (5). Because single antigen binding Fab fragments do not cause apoptosis, it was argued that toxicity involves signaling induced by cross-linking of PrP^C and that this may also underlie prion toxicity (5).

To investigate our candidate therapeutic antibodies, we stereotactically injected ICSM18 and ICSM35, fully humanized ICSM18 (both IgG1 and G4 isotypes), and their isotype controls into the hippocampus of C57BL/10 mice. ICSM35 recognizes PrP epitope 93 to 105 (6), whereas ICSM18 binds to 143 to 153 (7). We injected IgG P and IgG D13 as positive controls, as well as the nonapoptotic IgG D18 into both C57BL/10 mice and FVB mice. Antibody IgG b12, raised against the HIV-1 envelope glycoprotein gp120, was injected into the contralateral hippocampus as a negative control. We injected 2 μl antibodies against PrP (1 mg/ml) according to the published protocol (5) and killed the mice 48 hours after injection. Bilateral injection sites were histologically identified in all brains. We only analyzed specimens with an entirely visible injection track in the hippocampus. On average 70% of stereotaxic injections were on target, and at least four brains per monoclonal antibody were analyzed. Many of the injection sites showed local acute hemorrhage of variable size (Fig. 1 and figs. S1 and S2). Adjacent to the injection sites, acutely hypoxic and occasionally necrotic neurones were detected, which we considered a result of direct mechanical damage. The previously described (5) widespread apoptotic cell death through-

out the hippocampal region was not observed with antibodies IgG D13 and IgG P in C57BL/10 mice (Fig. 1) or in FVB mice (fig. S1). Likewise, injections with ICSM18 and ICSM35 failed to generate widespread neuronal apoptosis in the hippocampus of C57BL/10 mice (Fig. 1). To ensure that the negative histology results were not due to loss of activity

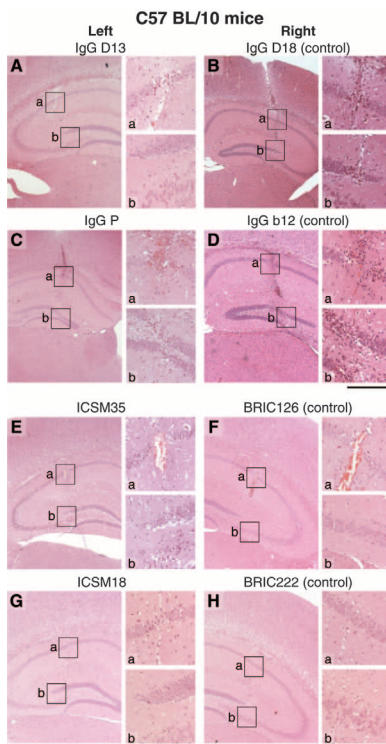


Fig. 1. No apoptosis after hippocampal injection of PrP antibodies in C57BL/10 mice. Antibodies IgG D13 (A), IgG P (C), ICSM35 (E), and ICSM18 (G) were injected into the left hippocampus. Negative control antibodies IgG D18 (B), IgG b12 (D), and isotype controls BRIC126 (F) and BRIC222 (H) were injected contralaterally. Needle track (Aa) or small hemorrhage (Ca) in CA1 region. The dentate gyrus remained unharmed (Ab) or showed mechanical damage (Cb). No apoptosis was observed, even in the immediate vicinity of the needle track. Injection track in dentate gyrus and CA1 region: Focal cell death because of mechanical damage, but no apoptosis around the injection sites (Ba, Bb, Da, and Db). Small needle track and minimal hemorrhage after ICSM35 and ICSM18 injection but no apoptosis in the CA1 region (Ea and Ga) or dentate gyrus (Eb and Gb). Injections with isotype controls BRIC126 (F) and BRIC222 (H) resulted in needle track lesion in CA1 but no apoptosis (Fa, Fb, Ha, and Hb). Scale bars indicate 900 μm in the main images and 300 μm in the magnified images.

of the proapoptotic antibodies, we performed an enzyme-linked immunosorbent assay (ELISA) to demonstrate PrP antigen recognition of the antibodies IgG D13, IgG P, and IgG D18 (fig. S6), and all recognized recombinant α-PrP at antibody concentrations of at least 1 ng/ml. To further confirm the functional integrity of the PrP antibodies we confirmed their known ability to cure prion-infected cells (7, 8) (fig. S7). We injected fully humanized versions of ICSM18, huICSM18 IgG4, and huICSM18 IgG1 into the hippocampus of C57BL/10 mice, which failed to show apoptosis (fig. S2). We also performed immunostaining for cleaved caspase 3 on consecutive sections to those shown in Fig. 1 and figs. S1 and S2, which showed no evidence that these antibodies elicited apoptosis (figs. S3 to S5).

The reason for the marked discrepancy with results of the previous study is unclear. We included IgG D13 and IgG P as positive controls, but found no evidence for significant apoptosis while confirming that all antibodies retained their potency of PrP binding and cell curing. Thus, antibodies targeting PrP, including the region 95 to 105, may be injected into the mouse brain at least at concentrations of up to 1 mg/ml without causing extensive apoptosis or other histologically apparent injury other than that expected from injection trauma. Consequently, toxicity from putative antibody-mediated cross-linking of cell-surface PrP^C is unlikely to limit therapeutic trial of PrP antibodies in these rapidly progressive and invariably fatal neurodegenerative diseases. In addition, our data do not support a model in which neuronal cell death in prion disease is mediated by cross-linking of PrP^C by disease-related PrP aggregates.

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Supporting Online Material

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Figs. S1 to S7

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The Technology Path to Deep Greenhouse Gas Emissions Cuts by 2050: The Pivotal Role of Electricity

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Several states and countries have adopted targets for deep reductions in greenhouse gas emissions by 2050, but there has been little physically realistic modeling of the energy and economic transformations required. We analyzed the infrastructure and technology path required to meet California's goal of an 80% reduction below 1990 levels, using detailed modeling of infrastructure stocks, resource constraints, and electricity system operability. We found that technically feasible levels of energy efficiency and decarbonized energy supply alone are not sufficient; widespread electrification of transportation and other sectors is required. Decarbonized electricity would become the dominant form of energy supply, posing challenges and opportunities for economic growth and climate policy. This transformation demands technologies that are not yet commercialized, as well as coordination of investment, technology development, and infrastructure deployment.

In 2004, Pacala and Socolow (1) proposed a way to stabilize climate using existing greenhouse gas (GHG) mitigation technologies, visualized as interchangeable, global-scale “wedges” of equivalent emissions reductions. Subsequent work has produced more detailed analyses, but none combines the sectoral granularity, physical and resource constraints, and geographic scale needed for developing realistic technology and policy roadmaps (2–4). We addressed this gap by analyzing the specific changes in infrastructure, technology, cost, and governance required to decarbonize a major economy, at the state level, that has primary jurisdiction over electricity supply, transportation planning, building standards, and other key components of an energy transition.

California is the world's sixth largest economy and 12th largest emitter of GHGs. Its per capita GDP and GHG emissions are similar to those of Japan and western Europe, and its policy and technology choices have broad relevance nationally and globally (5, 6). California's Assembly Bill 32 (AB32) requires the state to reduce GHG emissions to 1990 levels by 2020, a reduction of 30% relative to business-as-usual assumptions (7). Previous modeling work we performed for California's state government formed the analytical foundation for the state's AB32 implementation plan in the electricity and natural gas sectors (8, 9).

California has also set a target of reducing 2050 emissions 80% below the 1990 level, con-

sistent with an Intergovernmental Panel on Climate Change (IPCC) emissions trajectory that would stabilize atmospheric GHG concentrations at 450 parts per million carbon dioxide equivalent (CO₂e) and reduce the likelihood of dangerous anthropogenic interference with climate (10). Working at both time scales, we found a pressing need for methodologies that bridge the analytical gap between planning for shallower, near-term GHG reductions, based entirely on existing commercialized technology, and deeper, long-term GHG reductions, which will depend substantially on technologies that are not yet commercialized.

We used a stock-rollover methodology that simulated physical infrastructure at an aggregate level, and built scenarios to explore mitigation options (11, 12). Our model divided California's economy into six energy demand sectors and two energy supply sectors, plus cross-sectoral economic activities that produce non-energy and non-CO₂ GHG emissions. The model adjusted the infrastructure stock (e.g., vehicle fleets, buildings, power plants, and industrial equipment) in each sector as new infrastructure was added and old infrastructure was retired, each year from 2008 to 2050. We constructed a baseline scenario from government forecasts of population and gross state product, combined with regression-based infrastructure characteristics and emissions intensities, producing a 2050 emissions baseline of 875 Mt CO₂e (Fig. 1). In mitigation scenarios, we used backcasting, setting 2050 emissions at the state target of 85 Mt CO₂e as a constrained outcome, and altered the emissions intensities of new infrastructure over time as needed to meet the target, employing 72 types of physical mitigation measures (13). In the short term, measure selection was driven by implementation plans for AB32 and other state policies (table S1). In the long term, technological progress and rates of introduction were constrained by physical feasi-

bility, resource availability, and historical uptake rates rather than relative prices of technology, energy, or carbon as in general equilibrium models (14). Technology penetration levels in our model are within the range of technological feasibility for the United States suggested by recent assessments (table S20) (15, 16). We did not include technologies expected to be far from commercialization in the next few decades, such as fusion-based electricity. Mitigation cost was calculated as the difference between total fuel and measure costs in the mitigation and baseline scenarios. Our fuel and technology cost assumptions, including learning curves (tables S4, S5, S11, and S12, and fig. S29), are comparable to those in other recent studies (17). Clearly, future costs are very uncertain over such a long time horizon, especially for technologies that are not yet commercialized. We did not assume explicit life-style changes (e.g., vegetarianism, bicycle transportation), which could have a substantial effect on mitigation requirements and costs (18); behavior change in our model is subsumed within conservation measures and energy efficiency (EE).

To ensure that electricity supply scenarios met the technical requirements for maintaining reliable service, we included an electricity system dispatch algorithm that tested grid operability. Without a dispatch model, it is difficult to determine whether a generation mix has infeasibly high levels of intermittent generation. We developed an electricity demand curve bottom-up from sectoral demand, by season and time of day. On the basis of the demand curve, the model constrained generation scenarios to satisfy in succession the energy, capacity, and system-balancing requirements for reliable operation. The operability constraint set physical limits on the penetration of different types of generation and specified the requirements for peaking generation, on-grid energy storage, transmission capacity, and out-of-state imports and exports for a given generation mix (table S13 and figs. S20 to S31). It was assumed that over the long run, California would not “go it alone” in pursuing deep GHG reductions, and thus that neighboring states would decarbonize their generation such that the carbon intensity of imports would be comparable to that of California in-state generation (19).

Electrification required to meet 80% reduction target. Three major energy system transformations were necessary to meet the target (Fig. 2). First, EE had to improve by at least 1.3% year⁻¹ over 40 years. Second, electricity supply had to be nearly decarbonized, with 2050 emissions intensity less than 0.025 kg CO₂e/kWh. Third, most existing direct fuel uses had to be electrified, with electricity constituting 55% of end-use energy in 2050 versus 15% today. Results for a mitigation scenario, including these and other measures, are shown in Fig. 1. Of the emissions reductions relative to 2050 baseline emissions, 28% came from EE, 27% from decarbonization of electricity generation, 14% from a combination of energy

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conservation and alternative energy measures [including “smart growth” urban planning, bio-fuels, and rooftop solar photovoltaics (PV)], 15% from measures to reduce non-energy CO₂ and non-CO₂ GHGs, and 16% from electrification of existing direct fuel uses in transportation, buildings, and industrial processes. Table 1 shows changes from 2010 to 2050 in primary and end-use energy and emissions by sector and fuel type for the baseline and mitigation cases, along with per capita and economic intensity metrics.

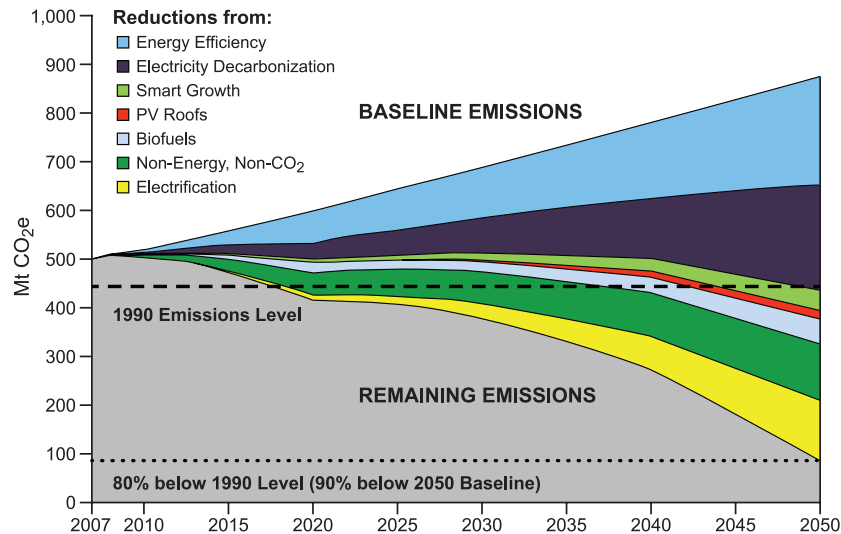
The most important finding of this research is that, after other emission reduction measures were employed to the maximum feasible extent, there was no alternative to widespread switching of direct fuel uses (e.g., gasoline in cars) to electricity in order to achieve the reduction target. Without electrification, the other measures combined produced at best 2050 emissions of 210 Mt CO₂e, about 50% below the 1990 level. The largest share of GHG reductions from electrification came from transportation, in which 70% of vehicle miles traveled—including almost all light-duty vehicle miles—were powered by electricity in 2050, along with 20% powered by biofuels and 10% powered by fossil fuels. Other key applications for fuel switching occurred in space heating, water heating, and industrial processes. Figure 3A shows that even with aggressive EE keeping other demand growth nearly flat, fuel-switching to electricity led to a doubling of electricity generation by 2050. “Smart charging” of electric vehicles was essential for reducing the cost of electrification, by raising utility load factors and reducing peak capacity requirements through automated control of charging times and levels (Fig. 3B).

In the electricity sector, three forms of decarbonized generation—renewable energy, nuclear, and fossil fuel with carbon capture and storage (CCS)—each have the potential to become the principal long-term electricity resource in California, given its resource endowments. All currently suffer from technical limitations and high cost relative to the conventional generation alternative, natural gas, so it is not obvious which of these (if any) will dominate in the long run. Therefore, we built separate high-renewable energy, high-nuclear, and high-CCS scenarios that met the target, plus a mixed case. Because these technologies have very different operating characteristics—CCS, when commercialized, is expected to be flexibly dispatchable generation that can follow demand; nuclear is baseload generation that operates at a constant output level; and the most abundant renewable energy resources (wind and solar) are intermittent—they also have very different needs for supporting infrastructures, including capacity resources, high-voltage transmission, and energy storage. Figure 3C shows the generation scenarios. The high-renewable energy case has the highest requirements for installed capacity, transmission, and energy storage; the high-nuclear case requires the largest export market for excess generation, along with an expansion of upstream and downstream nuclear fuel cycle

infrastructure; and the high-CCS case requires construction of CO₂ transportation and storage infrastructure. In addition, water, land use, and siting issues are quite different for each of these options. Residual electricity-sector carbon emissions in 2050 came primarily from combustion of natural gas for peaking generation and CCS. CCS fleet-average carbon storage efficiency in 2050 was 90%, but new CCS units were required to reach 98% efficiency. Within the western grid of which California is part, all existing conventional coal plants were retired at the end of their planning lives of 30 years.

Some studies suggest that 100% of future electricity requirements could be met by renew-

able energy, but our analysis found this level of penetration to be infeasible for California (20, 21). We found a maximum of 74% renewable energy penetration despite California’s large endowment of renewable resources, even assuming perfect renewable generation forecasting, breakthroughs in storage technology, replacement of steam generation with fast-response gas generation, and a major shift in load curves by smart charging of vehicles. Using historical solar and wind resource profiles in California and surrounding states, the electricity system required 26% nonrenewable generation from nuclear, natural gas, and hydro-electricity, plus high storage capacity to maintain operability. It would be possible to forecast higher



Wedge Category:	Emissions Reduction Mt CO ₂ e (% of Total)		Types (and Numbers) of Measures Used	Key Attributes in 2050
	2030	2050		
Energy Efficiency	102 (33%)	223 (28%)	Building EE (18); Vehicle EE (9); Other EE (6)	Energy efficiency improved 1.3% per year on average for 40 years
Electricity Decarbonization	72 (23%)	217 (27%)	High renewables, high nuclear, high CCS, and mixture of the three	90% of generation requirement met with CO ₂ -free sources. Equivalent decarbonization in each scenario
Smart Growth	13 (4%)	41 (5%)	Reductions in vehicle miles traveled (VMT) (6)	VMT reduced in light duty vehicles (LDV) by 10%; freight trucks 20%; other transportation 20%
Rooftop PV	8 (3%)	21 (3%)	Residential and commercial PV roofs (2)	10% of electricity demand displaced by rooftop PV
Biofuels	18 (6%)	49 (6%)	Transportation biofuels; ethanol, biodiesel, biojet fuel (9); Residential, commercial, industrial biomethane (3)	2% of natural gas use in buildings displaced by biomethane, and 10-20% of petroleum-based fuels for vehicles displaced by biofuels
Non-Energy, Non-CO ₂	67 (22%)	116 (15%)	Cement, agriculture, and other (3)	Non-fuel, non-CO ₂ GHG emissions reduced 80% below baseline
Electrification	29 (9%)	124 (16%)	Transportation electrification (9); Other end-use electrification (5)	75% of LDV gasoline use displaced by PHEVs & electric vehicles; 30% of fuel use in other transport sectors electrified; 65% electrification of non-heating/cooling fuel use in buildings; 50% electrification of industrial fuel uses
Baseline Case Emissions	688	875		
Mitigation Case Emissions	380	85		
Total Reduction	308	790		

Fig. 1. Emission reduction wedges for California in 2050. **Top:** Measures grouped into seven “wedges” reduce emissions from 875 Mt CO₂e in the 2050 baseline case to 85 Mt CO₂e in the mitigation case. In the 2020 model results, the wedge contributions are consistent with implementation plans for California’s policy objectives (AB32) for 2020. **Bottom:** Reductions by wedge are shown for the 2030 and 2050 mitigation cases, in Mt CO₂e and as a percentage of total reductions. The top three contributions are from energy efficiency (EE) (28%), electricity decarbonization (27%), and electrification of direct fuel uses (16%). For each wedge, the types of measures included and key assumptions are shown.

penetration in cases with a higher resource base and/or much lower energy demand—for example, as a result of lower population growth or lower economic growth.

Unprecedented energy efficiency; limited contribution from biofuels. The rate of EE improvement required to achieve the target and enable feasible levels of decarbonized generation and electrification—1.3% year⁻¹ reduction relative to forecast demand—is less than the level California achieved during its 2000–2001 electricity crisis (22) but is historically unprecedented over a sustained period. This level is, however, consistent with the upper end of estimates of long-term technical EE potential in recent studies (23, 24). In our model, the largest share of GHG reductions from EE came from the building sector, through a combination of efficiency improvements in building shell, HVAC systems, lighting, and appliances. EE improvements were complemented by other measures to reduce new energy supply requirements for electricity, transportation, and heating. EE in combination with on-site distributed energy resources (in the form of solar hot water and rooftop PV) reduced the net consumption of grid-supplied electricity and fuels in new residential and commercial buildings to zero by 2030 (25). Structural conservation in the form of “smart growth” urban planning to reduce driving require-

ments was responsible for 5% of total emission reductions in 2050.

Biofuels, although essential (because not all transportation can be electrified), made only a modest 6% contribution to the 2050 emissions reduction when feedstocks were constrained to be carbon-neutral, produced in the United States, and limited to California’s consumption-weighted proportional share of U.S. production (26–28). This feedstock was sufficient to provide 20% of transportation fuels in the form of cellulosic ethanol and algal biodiesel, assuming that these technologies achieve commercialization (fig. S15). In our model, biofuel feedstocks were dedicated to the production of transportation fuels as their highest-valued economic use, and these fuels were allocated to applications for which electrification is not a practical option, such as long-haul freight trucking and air travel. A small amount of biomethane was used in power generation.

In the baseline forecast, 2050 emissions of non-energy CO₂ (e.g., from cement manufacturing) and non-CO₂ GHGs [e.g., methane and nitrous oxide from agriculture and waste treatment, and high-global warming potential (GWP) gases used as refrigerants and cleaning agents] were 145 Mt CO₂e, more than the entire economy-wide target of 85 Mt CO₂e. Relative to CO₂ emissions from energy sectors, scientific understanding of

long-term mitigation potential for these sectors is poorly developed (29–32). Nevertheless, it was clear that if these emissions were not abated, the 2050 target could not be met. We modeled mitigation by extrapolating California’s AB32 implementation plan for 2020 (7) in three broad areas. Agricultural and forestry measures contributed 47 Mt CO₂e of reductions, cement-related measures contributed 8 Mt CO₂e, and industrial and other measures contributed 61 Mt CO₂e, for a total reduction of 116 Mt CO₂e below the 2050 baseline, which maintained the current share of non-energy/non-CO₂ in overall emissions.

There is evidence that the three key energy system transformations identified here are broadly generalizable to developed economies. A recent report on 80% GHG reductions in the European Union found that similar transformations were required, including electrification of transportation and buildings (33). In other studies, where reductions rely on EE and generation decarbonization but not electrification, lower GHG reduction levels were achieved. For example, in a recent International Energy Agency study of technology paths in Organization for Economic Cooperation and Development member countries as a whole, the most aggressive scenario had a 2050 reduction of about 50% below 1990 levels, with a 6% contribution from electrification (34). The consistency among these results is predictable, in that developed economies broadly share the same challenges for reaching deep reduction targets—the need to virtually eliminate fossil fuel use in electricity supply and in final consumption, especially in vehicles and buildings.

Infrastructure deployment and technology investment require coordination. In contrast to the findings of Pacala and Socolow, we found that achieving the infrastructure changes described above will require major improvements in the functionality and cost of a wide array of technologies and infrastructure systems, including but not limited to cellulosic and algal biofuels, CCS, on-grid energy storage, electric vehicle batteries, smart charging, building shells and appliances, cement manufacturing, electric industrial boilers, agriculture and forestry practices, and source reduction/capture of high-GWP emissions from industry (35).

Not only must these technologies and systems be commercially ready, they must also be deployed in a coordinated fashion to achieve their hoped-for emission reduction benefits at acceptable cost. For example, switching from fuels to electricity before the grid is substantially decarbonized negates the emissions benefits of electrification; large-scale deployment of electric vehicles without smart charging will reduce utility load factors and increase electricity costs; and without aggressive EE, the bulk requirements for decarbonized electricity would be doubled, making achievement of 2050 goals much more difficult in terms of capital investment and siting. Figure 3D shows the impact of aggressive EE on three key metrics of decarbonized electricity supply:

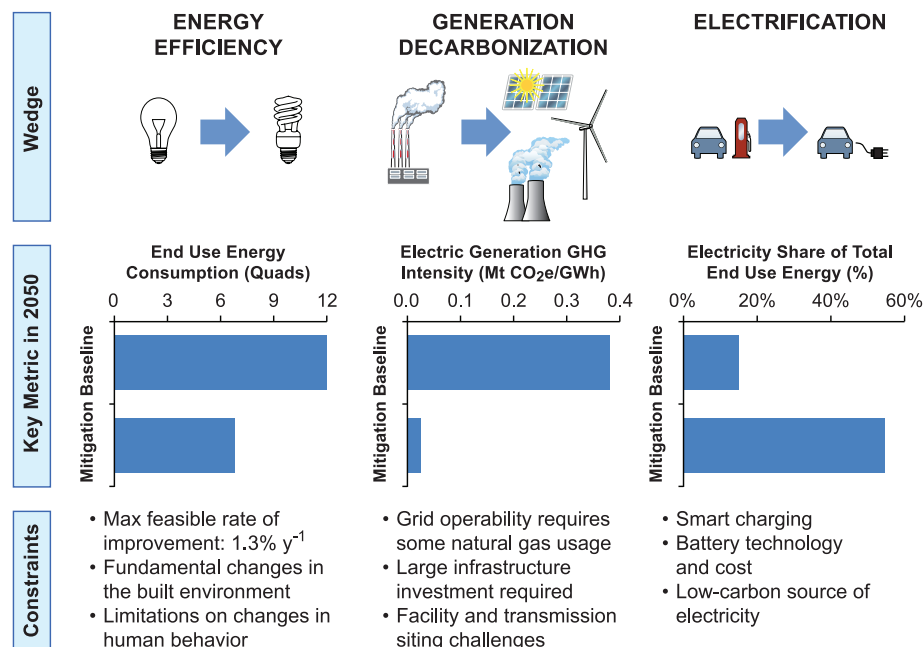


Fig. 2. The three main energy system transformations required to reduce GHG emissions 80% below 1990 levels by 2050 in California. End-use EE must be improved very aggressively (annual average rate 1.3% year⁻¹), electric generation emissions intensity must be reduced to less than 0.02 kg CO₂e/kWh, and most direct fossil fuel uses in transport, buildings, and industry must switch to electricity, raising the electricity share of end-use energy from 15% today to 55% in 2050. Both economics and the current state of technology development suggest a staged deployment in large-scale infrastructural transformation. Without aggressive levels of EE, the scale of decarbonized generation required to simultaneously replace fossil plants and meet both existing and newly electrified loads would be infeasible. Until high levels of electricity decarbonization are achieved, emission benefits from electrification would be limited. Without electrification, constraints on the other measures would limit total reductions to about 50% below 1990 levels.

generating capacity, energy storage, and miles of high-voltage transmission line. For the mixed-generation case, achieving the 2050 target with baseline levels of EE raised the requirement for annual construction of decarbonized generation from a very formidable 3.7 GW year^{-1} to a practically unachievable 7.0 GW year^{-1} , and the requirement for new transmission lines from 400 to 960 miles year^{-1} .

Our model shows a net mitigation cost to California, relative to the baseline, of 0.5% of gross state product (GSP) in 2020, 1.2% in 2035, and 1.3% in 2050 (\$65 billion or \$1200 per capita) (Fig. 4 and fig. S34). The transportation sector bore the highest share of these costs, reflecting

the cost of fleet electrification. These results are highly sensitive to both measure costs and fuel price assumptions; using the upper value of the U.S. Energy Information Administration (EIA) long-term crude oil price forecast makes net mitigation costs negative (fig. S12). Cumulative net costs from 2010 to 2050 were \$1.4 trillion. The average cost of carbon in 2050 was \$90/t CO_2e , whereas the highest average cost by measure type was \$600/t CO_2e for electrification measures (36). Because mitigation measures reduce fuel use by investing in energy-efficient infrastructure and low carbon generation, a much higher percentage of energy cost will go to capital costs; our model indicates a cumulative investment of \$400 billion

to \$500 billion in current dollars (figs. S35 and S36) for electricity generation capacity in the mitigation case, a factor of about 10 higher than the baseline case (37).

The transition to an energy-efficient, low-carbon, electrified infrastructure thus requires mobilizing investment and coordinating technology development and deployment on a very large scale over a very long time period. How best to achieve this is the focus of active debate over the relative roles of markets, government, carbon pricing, R&D policy, regulation, and public investment (38). Many consider carbon pricing the key to achieving efficient investment and providing incentives for consumer adoption; others argue that carbon

Table 1. Primary and end-use energy and emissions by sector and fuel type in 2010 and 2050. The numerical difference between primary and end-use energy is due to conversion and other losses. Sources for population and economic data are given in the supporting online material.

	Energy consumption (EJ)					Emissions (Mt CO_2e)		
	2010	2050 Baseline	2050 Mitigation	2010 (%)	2050 Mitigation (%)	2010	2050 Baseline	2050 Mitigation
Primary energy consumption and emissions, by sector								
Residential	1.60	2.56	0.52	18%	8%	71.3	117.1	5.4
Commercial	1.68	2.60	0.94	19%	14%	70.9	114.5	10.0
Industrial	1.41	1.39	0.96	16%	14%	67.4	67.3	6.4
Petroleum	0.81	0.82	0.58	9%	9%	46.7	47.5	5.6
Agriculture	0.34	0.52	0.21	4%	3%	16.3	27.1	1.0
Transportation	2.86	5.67	3.60	33%	53%	189.4	374.1	45.0
Non-energy, non- CO_2 GHG emissions						56.4	127.8	11.4
Total all sectors	8.70	13.56	6.81	100%	100%	518.4	875.4	84.8
Primary energy consumption and emissions, by fuel type								
Direct fuel use								
Natural gas	2.73	3.40	0.38	31%	6%	148.9	185.1	20.5
Gasoline	2.09	4.36	0.13	24%	2%	135.9	283.4	8.3
Diesel	0.73	1.23	0.39	8%	6%	50.2	84.7	26.6
Jet fuel	0.04	0.08	0.04	0%	1%	3.3	6.0	3.4
Biomethane and biofuels	0.00	0.00	0.73	0%	11%	0.0	0.0	0.0
Total direct fuel use	5.59	9.06	1.67	64%	25%	338.3	559.2	58.8
Electric generation (primary)								
Natural gas (non-CCS)	1.45	2.90	0.01	17%	0%	72.1	135.3	0.4
Coal (non-CCS)	0.49	0.49	0.00	6%	0%	43.2	43.2	0.0
Fossil fuel w/ CCS	0.00	0.00	2.18	0%	32%	0.0	0.0	10.6
Nuclear	0.30	0.26	0.74	3%	11%	0.0	0.0	0.0
Renewables and hydroelectricity	0.71	0.66	2.04	8%	30%	0.4	0.4	0.8
Other	0.16	0.18	0.16	2%	2%	8.0	9.6	2.9
Total electric generation	3.11	4.49	5.14	36%	75%	123.7	188.4	14.7
Non-energy, non- CO_2 GHG emissions						56.4	127.8	11.4
Total all fuel types	8.70	13.56	6.81	100%	100%	518.4	875.4	84.8
End-use energy consumption and emissions, by fuel type								
Total direct fuel use	5.59	9.06	1.67	85%	45%	338.3	559.2	58.8
Electricity (end-use)	0.98	1.63	2.03	15%	55%	123.7	188.4	14.7
Direct fuel use + electricity	6.57	10.69	3.70	100%	100%	462.0	747.6	73.4
Non-energy, non- CO_2 GHG emissions						56.4	127.8	11.4
Total end use by fuel type	6.57	10.69	3.70	100%	100%	518.4	875.4	84.8
Intensity metrics								
CA population (millions)	38.8	56.6	56.6					
Per capita energy use rate (kW/person)	7.1	7.5	3.8					
Per capita emissions (t CO_2e /person)	13.3	15.5	1.5					
Energy intensity (\$/GJ)	\$249	\$383	\$762					
Economic emissions intensity (kg CO_2e /\$)	0.239	0.169	0.016					
Electric emissions intensity (kg CO_2e /kWh)	0.42	0.39	0.02					

pricing is insufficient and requires complementary policies to address market failures, public goods, and coordination problems (16, 39, 40). Some make the specific case that pollution pricing is effective in encouraging technology adoption but not technological innovation (41, 42). Others are concerned that the venture capital model is mismatched with the scale and timeline of investment required for an energy transformation (43) and with the risks created by the need for multiple technologies to achieve commercialization in parallel

(44). These concerns have led to calls for novel public-private partnerships to address investment failures through government absorption of private capital risk (43) and to address coordination and sequencing through industry-government road-mapping (45).

Electricity's role in future energy costs and climate policy. Another model result deserving special attention is the expanded role of electricity, which increases from 15% to 55% of end-use energy, essentially switching places with pe-

troleum products, which fall from 45% to 15% (Table 1). If electricity does become the dominant component of the 2050 energy economy, the cost of decarbonized electricity becomes a paramount economic issue. Our results show that generation mixes dominated by renewable energy, nuclear, and CCS, in the absence of cost breakthroughs, would have roughly comparable costs, raising the present average cost of electricity generation by a factor of about 2—a result also noted by other researchers (17). These findings indicate that minimizing the cost of decarbonized generation should be a key policy objective. By some estimates, aggressive R&D policies could reduce the cost of low-carbon generation in the United States from 2020 to 2050 by about 40% or \$1.5 trillion (17).

For electrified transportation, the inherently higher efficiencies of electric drivetrains would still allow a net reduction in fuel costs even with electricity prices doubled and oil prices at \$100 per barrel, as well as shifting cash flows away from foreign oil imports toward domestic purchases of electricity. On the other hand, electrification of direct fuel uses will increase costs in the residential, commercial, and industrial sectors, especially for heating; hence, there is a need for EE and design of new infrastructure in these sectors to minimize lifecycle costs. Because much of the required technology and infrastructure for a basic transformation of the energy system is not yet commercialized, comparative lifecycle costs are highly uncertain. However, because decarbonized generation technologies are dominated by capital costs and are insensitive to oil and natural gas price volatility, an electrified economy would have a long-term cost stability that could lower investment risk and make the optimal level of EE more certain (46). Even varying measure costs from one-half to twice the nominal values in the mitigation scenario produced no more variation in overall energy system costs than did varying crude oil prices in the baseline scenario over the range in the EIA's long-term forecast (fig. S12).

The climate policy community has proposed a suite of policies to complement carbon pricing (e.g., EE standards, renewable energy standards, and R&D support) that reflect not only economic and technology goals but also sociopolitical considerations such as equity, local initiative, and adaptability (16). The central role of electricity in our results suggests the importance of electricity-sector governance as a tool of climate policy, but this has received relatively little attention until recently (47). Although some argue that regulation impedes innovation and increases implementation costs (43), state-level electricity regulation has existing tools for pursuing many climate policy goals, through both market mechanisms and direct regulation. Regulators can require that utilities procure renewable generation, limit carbon intensities, implement customer EE and distributed energy programs, and set retail electricity rates that encourage conservation and electric vehicle charging, internalize pollution costs, and allocate the

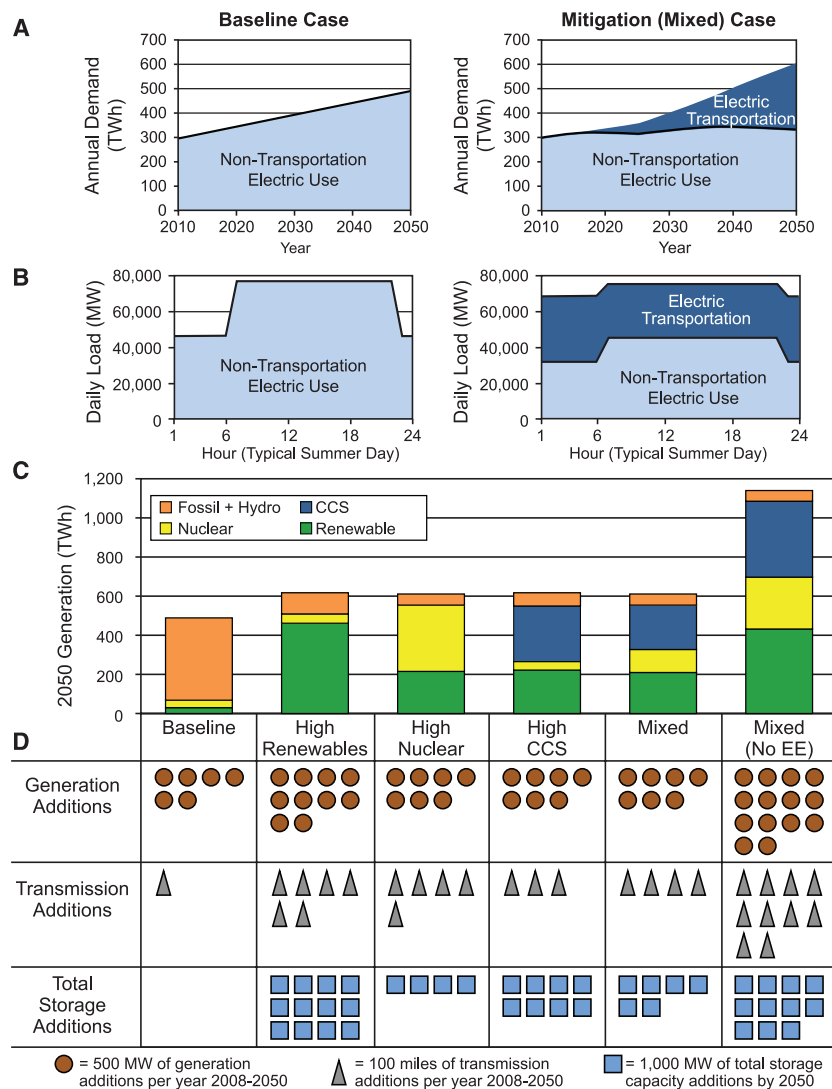


Fig. 3. Electricity consumption, load profiles, and fuel mix in baseline and mitigation scenarios. **(A)** In the mitigation case, aggressive end-use efficiency flattens baseline load growth. However, electrification of transportation adds a major new load, so that 2050 consumption is similar in both cases. **(B)** Smart charging of electric vehicles flattens the average daily load curve, reducing capacity requirements. **(C)** In the 2050 baseline scenario, load growth is met primarily by natural gas generation. Four mitigation scenarios are shown with different fuel mixes, constrained by California's existing fuel mix and policy requirements (e.g., 33% renewable portfolio standard, continued licensing of existing nuclear generation). The mixed case, which contains all three generation types, yields the results discussed in this paper and shown in Figs. 1 to 4. **(D)** New capacity requirements for each generation fuel mix are shown for generation, transmission, and energy storage. Without aggressive EE, new capacity requirements increase by roughly a factor of 2. The high-renewable energy case has higher new-capacity requirements than the high-CCS and high-nuclear cases; however, the high-renewable energy case does not have the high-CCS case requirements for CO₂ transmission and storage capacity, or the high-nuclear case requirements for upstream and downstream nuclear fuel cycle facilities.

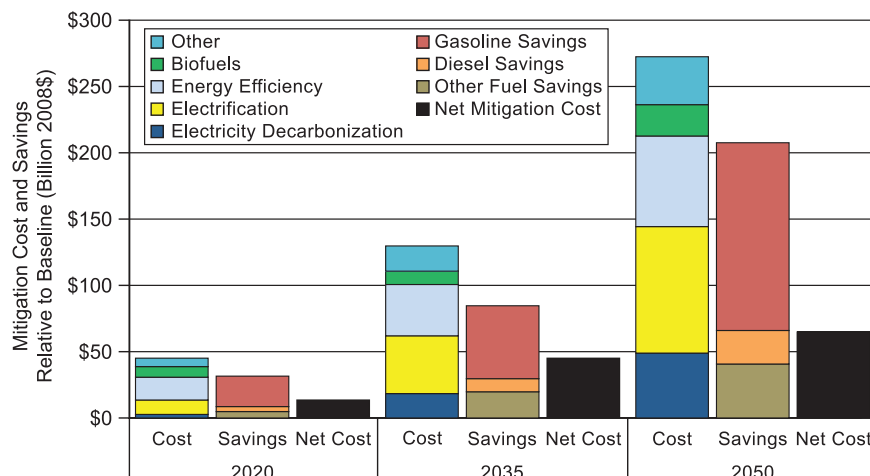


Fig. 4. Mixed-case net cost by mitigation type in 2020, 2035, and 2050. For each year shown, the left column shows incremental mitigation costs in excess of baseline costs, the center column shows incremental savings relative to baseline fuel costs, and the right column shows net cost (the difference between cost and savings). "Other" mixed-case costs include measure implementation costs not associated with EE, electrification, generation decarbonization, or biofuels. "Other" savings include jet fuel and natural gas purchases for direct use (e.g., heating). Net costs are \$15 billion in 2020, \$45 billion in 2035, and \$65 billion in 2050. This is equivalent to \$320 per capita or 0.5% of the statewide GSP in 2020, \$910 per capita or 1.2% of the statewide GSP in 2035, and \$1200 per capita or 1.3% of the statewide GSP in 2050.

costs of these policies equitably (7, 48). Given the political challenges of achieving comprehensive federal climate legislation, it is worth further exploring decentralized electricity governance as a climate policy mechanism.

Assuming plausible technological advances, we find that it is possible for California to achieve deep GHG reductions by 2050 with little change in life-style (although the potential for life-style change deserves further study). The logical sequence of deployment for the main components of this transformation is EE first, followed by decarbonization of generation, followed by electrification. This transformation will require electrification of most direct uses of oil and gas. In California, no single generation technology (renewable energy, nuclear, or CCS) can be used to decarbonize all electricity; a mixed generation portfolio is required. If it is true that the low-carbon path features electricity, then the question is how best to mobilize investment and coordinate R&D and infrastructure rollout to achieve this end, and what climate policy modalities will be most effective. If the oil economy is replaced by the electric economy, it is instructive to consider the implications of the price of a decarbonized kilowatt hour replacing the price of a barrel of oil as a benchmark for the overall economy.

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states, the timing of technology adoption in California is driven by policy as much as by markets, leading to the adoption of high-cost options (e.g., rooftop PV) concurrently with low-cost options (e.g., EE).

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REPORTS

Subparticle Ultrafast Spectrum Imaging in 4D Electron Microscopy

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Single-particle imaging of structures has become a powerful methodology in nanoscience and molecular and cell biology. We report the development of subparticle imaging with space, time, and energy resolutions of nanometers, femtoseconds, and millielectron volts, respectively. By using scanning electron probes across optically excited nanoparticles and interfaces, we simultaneously constructed energy-time and space-time maps. Spectrum images were then obtained for the nanoscale dielectric fields, with the energy resolution set by the photon rather than the electron, as demonstrated here with two examples (silver nanoparticles and the metallic copper–vacuum interface). This development thus combines the high spatial resolution of electron microscopy with the high energy resolution of optical techniques and ultrafast temporal response, opening the door to various applications in elemental analysis as well as mapping of interfaces and plasmonics.

Substantial progress has been made in the imaging of matter at the smallest length scale and shortest time response, using a range of optical and electron-based methods. Recent developments in electron microscopy have enabled studies of nanostructures with remarkable spectral and spatial resolution (1–4). However, a single nanoparticle-probing method with simultaneously high spatial, temporal, and spectral resolution has not hitherto been reported.

Here, we report ultrafast spectrum imaging (USI), with subparticle spatial resolution, in electron microscopy. The electron beam is focused

down to the nanometer scale, the electron packet has femtosecond duration, and the energy resolution, optically induced, is in the millielectron-volt range; the energy and temporal resolutions are no longer limited to those of conventional microscopy imaging. At every probe position across a nanoparticle, or at an interface, the electron energy-gain spectrum can be acquired as a function of the time delay between femtosecond optical and electron pulses, and imaging is complete when the focused probe is simultaneously scanned.

We conducted two sets of experiments to demonstrate the potential of the technique. For plasmonic Ag particles, we observed the polarized electric field distribution, the femtosecond dielectric response, and the nanometer spatial localization of a single particle. For the Cu metal–vacuum interface, we determined the effective decay length

(nanometer scale) and the evolution (femtosecond resolution) of the plasmonic field, and identified the strong and weak regions of the field by scanning the probe away from the interface. We anticipate a broad range of applications of USI because of the dimensions it simultaneously enables for imaging in space, time, and energy.

Knowledge of the dielectric response of materials and biological systems to an optical excitation is essential to the determination of the strength and extent of interaction between electromagnetic waves and systems under study. For example, bulk materials' reflection and absorption are dictated by such responses at the incident wavelengths. At the nanoscale, where the boundaries can have a marked effect on the way light manifests itself, the response can include spatially localized plasmonic fields (5, 6). It follows that an understanding of the dynamics at the microscopic level, with combined spatial, spectral, and temporal resolutions, would be indispensable, both at the fundamental level and for various applications.

For bulk systems, there exist various optical techniques for measuring the dielectric response; these include ellipsometry, Fourier transform infrared spectroscopy, and Raman spectroscopy. In the frequency domain, these techniques can readily reach the energy resolution necessary to differentiate vibrational and rotational modes in molecules (meV and sub-meV) and collective vibrational excitations in solids (phonons). In the spatial domain, however, these techniques are limited by diffraction effects, and hence they exhibit a typical resolution of several hundred nanometers at the visible wavelengths. Modern optical methods have enabled improvement of resolution (7–9) beyond the diffraction limit in certain circumstances, but they cannot provide the spatial resolution of

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electron microscopy, which is currently in the sub-angstrom regime (10, 11).

Convergent (focused) semirelativistic electrons, with their picometer wavelength, provide the means to study time-averaged images of single particles, molecules, and interfaces (12). When these probes are combined with the scanning and spectrometric capabilities of electron microscopes (13), detailed analysis of a specific energy loss with very high spatial precision is possible. The rich applications of this methodology in (sub)nanoscale science began with the mapping of charged states of silicon (14) and carbon atoms (15) across interfaces. Subsequently, single atoms inside nanotubes (16), solids (17), and atomic columns of crystals (18) were visualized with spectrum imaging of a specific core loss, and localized surface plasmons on a silver nanoparticle were recently mapped by using the low-loss region of the electron energy loss spectroscopy (EELS) data (19). The development of aberration-corrected microscopes has provided electron probes with enough sensitivity to achieve spectrum imaging with sub-angstrom resolution and sub-minute acquisition time (3).

Although the combination of real-space imaging and EELS formed the basis of a powerful technique, both the time and energy resolutions are still dictated by limitations of the microscopes used. The temporal resolution is controlled by the speed of the acquisition time of the detector (~30 ms), rendering many phenomena that oc-

cur on the fast/ultrafast time scale inaccessible to such microscopes. The energy resolution in EELS typically ranges from 0.4 to 1 eV, depending on the type of the electron gun used. With the advent of monochromated microscopes, it became possible to improve the resolution to 100 meV (20). Even with this impressive improvement, the energy resolution is still far below that of optical techniques. Moreover, the important visible-infrared spectral region is difficult to study, especially because this region is usually obscured by the tail of the large zero-loss peak (ZLP). The ideal imaging technique would therefore combine the spatial characteristics of electron microscopy with the spectrum characteristics of optical microscopy, at ultrafast temporal resolution.

Earlier work from our group has been concerned with the development of ultrafast electron microscopy and its applications to study phenomena such as structural dynamics and mechanical oscillations; these developments are summarized and detailed in (21, 22). More recently, adapting the ultrafast convergent beams (23) enabled Kikuchi diffraction (24) and dark-field real-space imaging (25). For near-field imaging, the work our group reported in (26) applied parallel-beam illumination with very wide energy selection; in contrast, here we invoke a focused scanning beam and energy is specified by the photon frequency. There were some concerns about the temporal resolution of a tightly focused ultrafast probe, because

the focused area is very small and space-charge effects may broaden the pulse. Here, we show that there is no loss of temporal resolution even in a 10-nm probe. The spectral resolution is set by the photons, not the electrons, as discussed below.

The USI setup is displayed schematically in Fig. 1. Following optical excitation, the femtosecond electron packets were focused onto a nanoparticle placed on a graphene substrate. At every spot position, a temporal scan was performed with step duration of 100 fs, and the electron energy gain and loss were recorded for every time step, relative to the time zero defined by the initial excitation. The probe was then scanned across and in the vicinity of the particle (see supporting online material). This enabled the formation of phase-space (time-energy) images as a function of the probe position and with subparticle spatial precision (Fig. 1, right). The data were then analyzed for all frames defined by their time, energy, and spatial coordinates to make USI movies, as shown in Fig. 2 and in movies S1 to S3. From these frames, it was possible to visualize the spatiotemporal dielectric response of the plasmonic particle after excitation with visible light. We emphasize that the observed behavior is at the exact pump energy of 2.4 eV, with the energy spread limited only by the few meV width of the initiating (pump) optical pulse. (In unpumped EELS studies, the energy resolution is typically 1 eV.) Moreover, when the pump-laser wavelength is

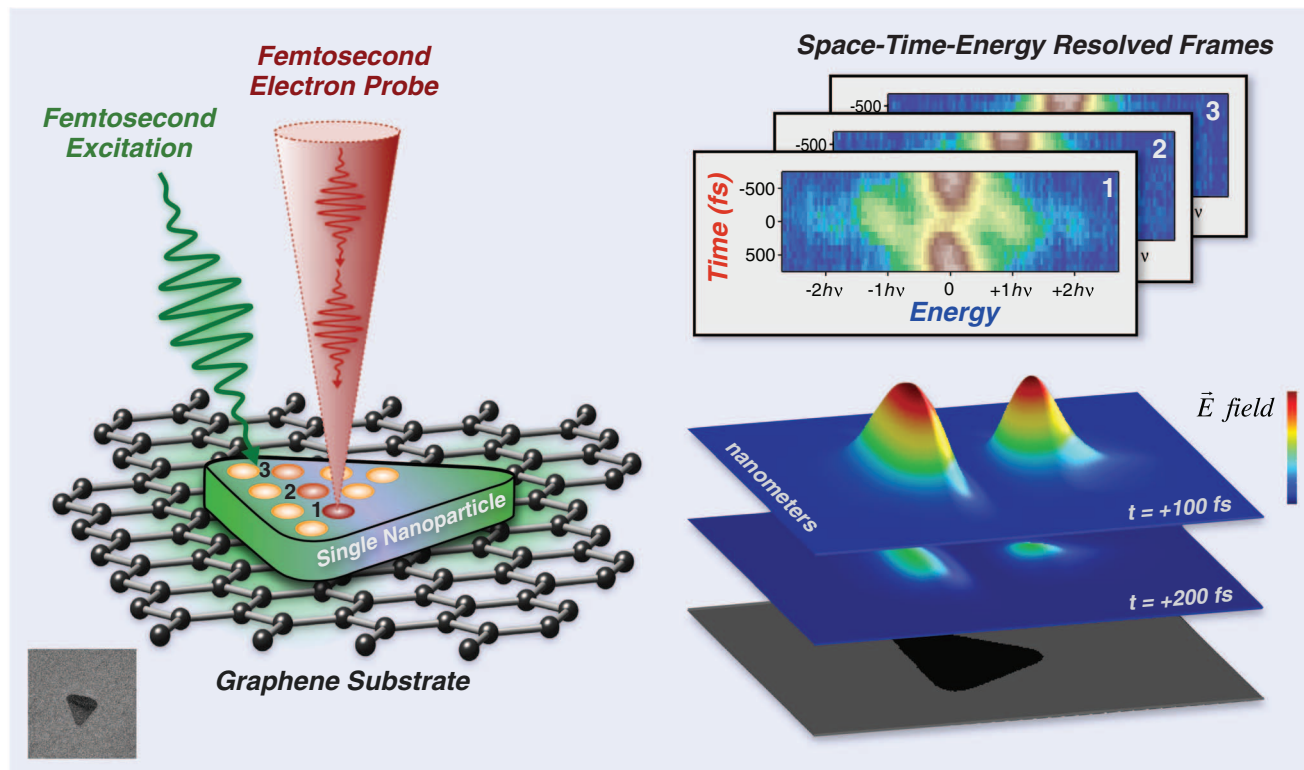


Fig. 1. Ultrafast spectrum imaging. A femtosecond nanoscale electron probe (here, 10 nm in diameter) is scanned across a graphene-supported plasmonic Ag nanoparticle that has been optically excited by a green laser pulse at 2.4 eV. At every probe position and femtosecond time delay, EEG spectra are acquired to

map the space-time-energy coordinates, as shown with typical frames at the upper right. Shown also are the bright-field image of the particle (with edge length 130 nm; lower left) and the field distributions in three dimensions together with projections at two time delays.

scanned, USI can map changes in the plasmonic fields, with the energy and spatial resolutions respectively set by optical and electron microscopy characteristics.

The inelastic loss and gain features seen in the energy-time images (Fig. 1, right; see below) at the multiples of the pump-laser energy ($\pm n\hbar\nu$, where n is the order number) are a direct result of photon exchange between the electron packets and the localized evanescent electric field of the particle (26). In the absence of external electromagnetic excitation, the inelastic interaction between electrons (pulsed or continuous) and matter exclusively involves energy loss. Once the structure is illuminated by external photons, the electrons may also gain energy from these photons, offsetting the collisional energy-loss phenomenon. Moreover, the cross section of electron energy gain (EEG) events depends on the pump laser fluence and is typically much higher than that of conventional energy-loss processes, such as those due to collective and single-valence electrons. This ultrafast phenomenon was experimentally observed in our laboratory (26) and was theoretically investigated by several groups (27–29). When it was invoked for real-space imaging, it was dubbed photon-induced near-field electron microscopy (PINEM).

Photons and near-relativistic electrons do not effectively couple in free space because the difference between their wave vectors (momenta) is large. One way to increase the coupling efficiency is to introduce a spatial confinement, or photon field distribution, along the direc-

tion of the electrons' propagation. Nanoscale objects with their localized fields provide the necessary momentum conservation requirement for electrons to effectively interact with photons through the reciprocity of the uncertainty principle ($\Delta x \Delta p \approx \hbar$). Because these interactions are inelastic, there is a force acting on the electron and the confined electric field will have a parallel component to the electrons' trajectory. In the classical picture, the process can be understood as acceleration (energy gain) and deceleration (energy loss) of electrons in the spatiotemporal field. It is these confined fields that USI explores.

In Fig. 2, the reconstructed USI frames at +200 fs and at time zero are shown for the gain of one quantum ($+1\hbar\nu$), two quanta ($+2\hbar\nu$), and no energy gain (i.e., zero-loss peak) (30). The probe position, diameter, and nanoparticle boundaries are all measured from the bright-field images and are shown to scale on the axes of Fig. 2. Examining the time-zero frame for $+1\hbar\nu$ mapping reveals that the strongest energy gains are localized at the left edge and at the right vertex of the triangular particle, with almost none occurring in the middle. The $+2\hbar\nu$ frame further confirms this observation. More important, because it is a two-photon gain ($n = 2$) process, the $+2\hbar\nu$ frame maps the electric field at its strongest locations, in analogy with pulse clipping, hence improving spatial localization. The field is concentrated around the apex, within an area ~ 20 nm in diameter, and at the opposite left edge, within an area ~ 40 nm in diameter. The ZLP frame shows a complementary

behavior to the energy-gain frames with reversed intensity distribution, as expected, although there is less observed localization because this frame contains contributions from all values of n . This detailed spatial behavior is accessible because of the resolution inherent in USI and its sensitivity to capturing images before the field decays on the femtosecond time scale.

After 200 fs have elapsed since excitation, the intensities of both one-photon and two-photon mapping have dropped as a result of the temporal response of the evanescent field and the excitation laser. The $+2\hbar\nu$ image diminishes faster than the $+1\hbar\nu$ image, rising and decaying in $\sigma = 220$ fs versus 290 fs for the first-order response. This improvement in temporal resolution of higher orders has been observed in PINEM studies (28). However, individual orders were not used for imaging because of the use of parallel-beam illumination and the large window of energy filtering. The temporal narrowing is understood because higher-order energy gains have a power-law dependence on the excitation pulse intensity, and hence they effectively reduce pulse duration; for instance, two-photon gain with a Gaussian pulse reduces the width by $\sqrt{2}$, in accordance with taking the square of the Gaussian. This improves the temporal resolution of the higher-order USI frames beyond the original pulses used.

The observed spatial behavior of the Ag nanoparticle can be qualitatively understood by first considering the linear polarization of the excitation light and the pertaining length scales. The triangular particle has a thickness of 20 nm

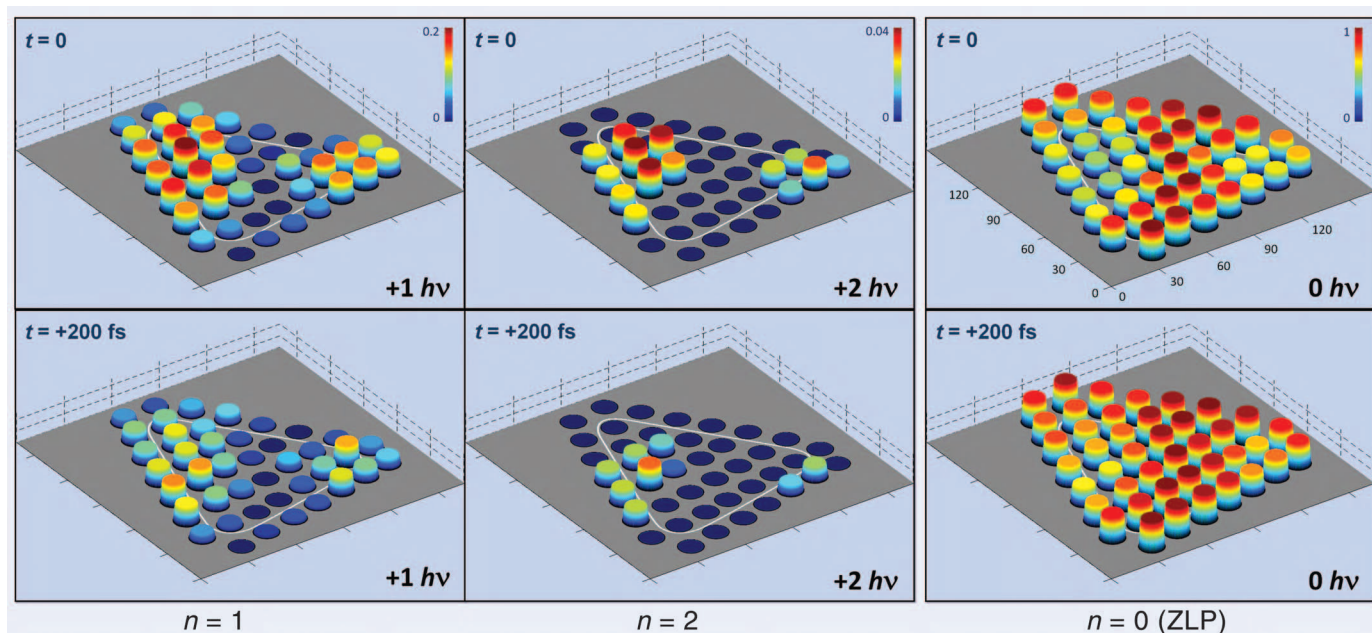


Fig. 2. USI time and order frames of the plasmonic triangular particle. Energy-gain images at $+1\hbar\nu$ (left), $+2\hbar\nu$ (center), and zero energy gain (right) are obtained for the Ag particle from the gain intensity at different time delays and probe positions (see Fig. 3A for energy gain spectra). The bottom row shows the temporal evolution after 200 fs, which maps the evanescent electric fields excited by the linearly polarized laser pulse. The intensity

behavior of the ZLP is reversed relative to energy-gain images (see text). The intensities are proportional to the height of the cylinders, which are mapped with false colors. Top and bottom rows share the same color scale designated by the bar. The spatial dimensions (in nanometers) in the lateral plane are indicated on the axes of the ZLP ($n = 0$) at $t = 0$ and are the same for all frames.

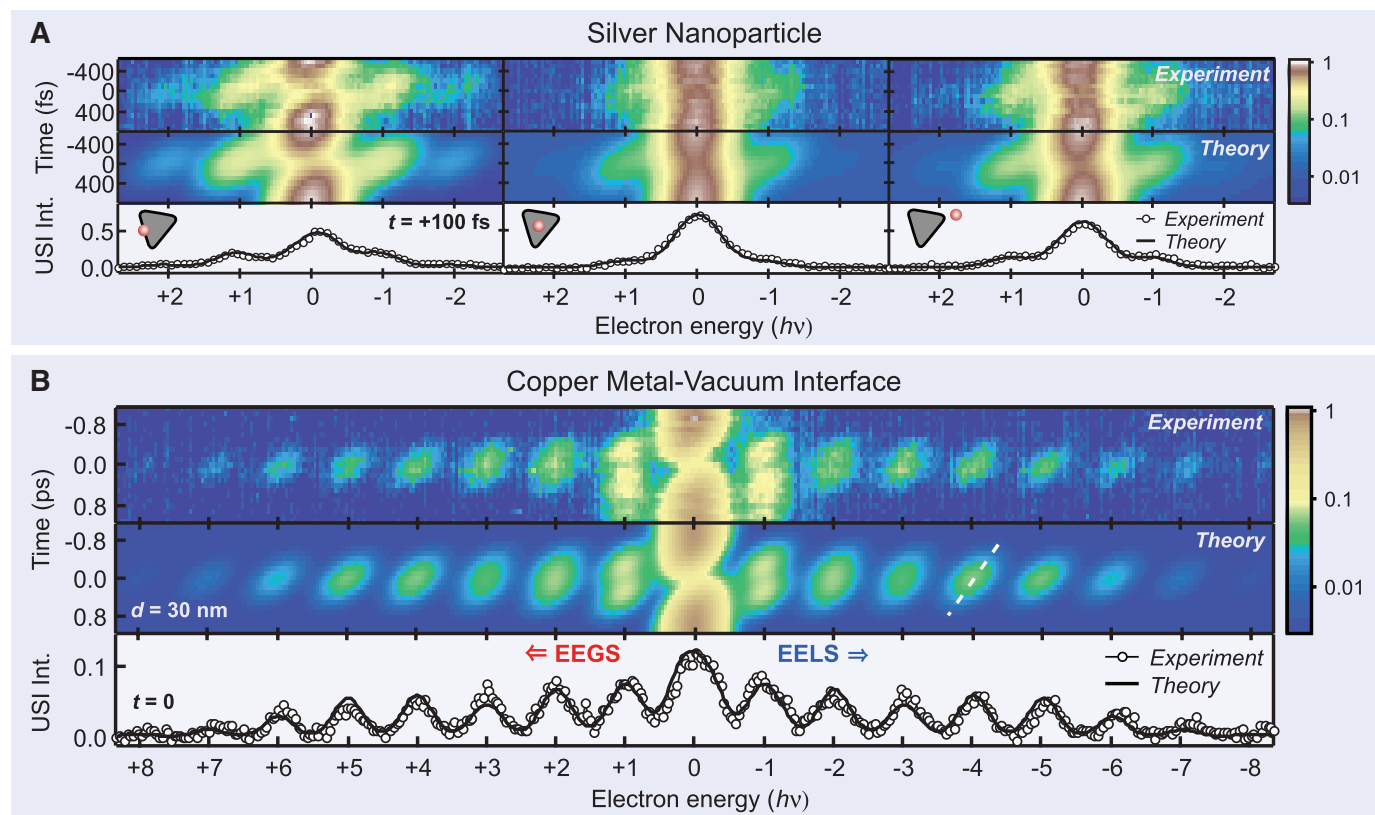


Fig. 3. (A) Experimental and theoretical phase-space (time-energy) images of the Ag nanoparticle as a function of probe position. A single spectrum at $t = +100$ fs is shown at the bottom of each image. The theory reproduces the experimental results very well; the time-dependent Schrödinger equation is used for the theoretical calculations without prior knowledge of the shape or strength of the field (see text). The false color scale is logarithmic; the schematic insets show the probe position relative to the particle in

space. **(B)** Phase-space images and time-zero energy spectrum (bottom) of the Cu metal-vacuum interface. The data were taken at 30 nm distance from the interface and in the vacuum. Seven energy-gain (EEGS) and energy-loss (EELS) orders, in units of $h\nu = 2.4$ eV, are clearly visible in both the experimental data and the theoretical calculations. The inclination with varying slopes for each order is indicated by a dashed white line. The theoretical treatment is distilled in the text.

(measured by low-loss EELS) and an edge length of 130 nm; these dimensions are smaller than the wavelength of the incident green photons (518 nm). This implies that the quasi-static approach of the Rayleigh limit can be invoked as a first-order approximation of light-matter interaction. In this regime, the conduction band charge density of the metallic particle exhibits a dipole-like behavior (for spherical particles) with a well-defined polarization direction, but the triangular shape may force charge redistribution toward the vertices through repulsion. These surface charge oscillations create an evanescent electric field (as described by Poisson's equation), which is imaged with USI, as shown in Fig. 2. The more complete Mie theory extends the dipolar behavior by including retardation effects and damping mechanisms (28).

To quantify the nature of the field, we performed theoretical calculations and compared the results with the experimental phase-space images for three different probe positions (Fig. 3A). The theory reproduces the experimental results remarkably well, further confirming that the strongest fields are close to the apex and edge of the particle and the weakest one is at the center of it. The theoretical calculations follow

the approach outlined in (28), where the time-dependent Schrödinger equation is solved for the three-body (electron-photon-nanostructure) interactions, giving an analytical relationship between the EEGS intensities and the electric fields involved. The experimentally measured EEGS signal is related to the integrated z -component of the electric field given by

$$U(\mathbf{r}_{xy}) = \frac{q_e}{h\nu} \left| \int_{-\infty}^{+\infty} E_z^*(z, \mathbf{r}_{xy}) \exp(-iz/b) dz \right| \quad (1)$$

where b , the characteristic impact parameter in EELS, is given by $h\nu_e/h\nu$; $h\nu$ is the photon energy of the excitation pulse; v_e is the speed of semirelativistic electrons; q_e is the unit electric charge; and E_z^* is the complex electric field parallel to the trajectory (z) of the electron. Here, we introduce the $\mathbf{r}_{xy}$ vector to account for the different probe positions that are in the x - y plane. It follows that if we use the USI results and a least-squares fit procedure, we can obtain the field strength $U(\mathbf{r}_{xy})$ as a function of probe position. These dimensionless quantities have the values $U(\mathbf{r}_1) = 1.88$, $U(\mathbf{r}_2) = 0.95$, and $U(\mathbf{r}_3) = 1.24$ for three different positions shown in Fig.

3A. Thus, without prior knowledge of the particle's shape or the excitation laser's polarization, the electric field can be quantified with subparticle spatial resolution.

The phenomena discussed so far involve weak electric fields. To go beyond this regime, we have also studied the Cu metal-vacuum interface, for which the plasmonic field is strong in the vicinity of the interface. Relative to the Ag nanoparticle, the Cu slab is semi-infinite in the lateral direction, and higher charge (and field) densities are therefore expected to accumulate at the interface upon excitation with linearly polarized light. Regions that were in close proximity to the interface were previously inaccessible, but with the convergent ultrafast electron probes, they can now be studied. Figure 4C depicts the probing arrangement of the interface. We note that cathodoluminescence can provide spectral responses with nanometer spatial resolution (31); however, it is limited to radiative emission modes and, more important, the processes are caused by electron impact, not photon excitation.

Unlike the case of the Ag nanoparticle, we observed two regimes of interaction at the Cu-vacuum interface, which we term "dynamical" and "kinematical" EEGS; these dominate in the

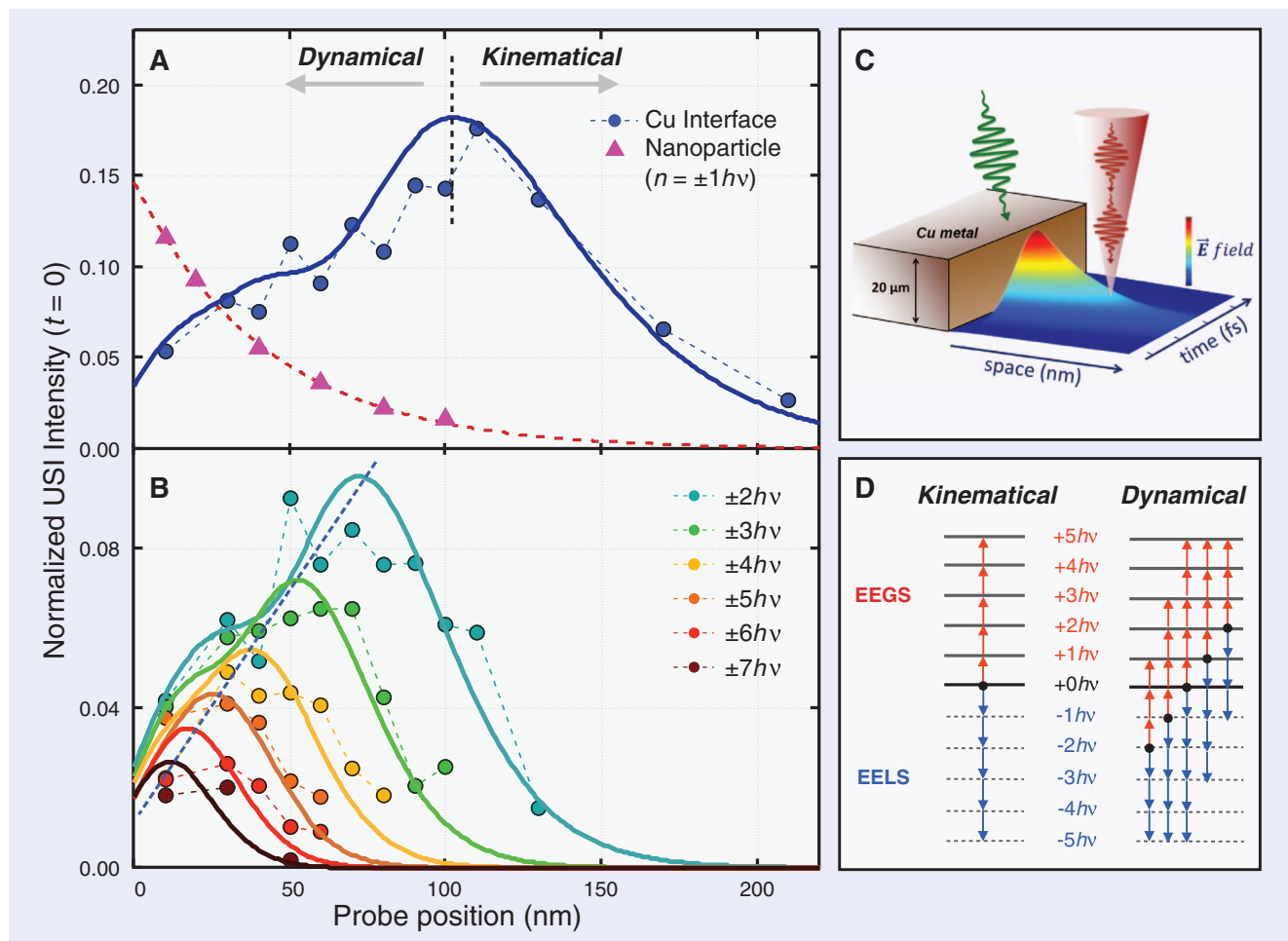


Fig. 4. Probe scans at the Cu metal–vacuum and, for comparison, Ag nanoparticle–vacuum interfaces. **(A)** Normalized USI intensity (at $t = 0$) versus probe distance from the interface for the two systems studied; note the distinct difference between the particle and slab behaviors. The Ag nanoparticle scan was taken outside and away from the highest-intensity edge of the particle. The red dashed line is an exponential fit to the particle data. **(B)** Intensity profile for the different orders studied; the dashed blue line illus-

trates the origin of spatial confinement as the order increases, as observed experimentally (circles) and calculated theoretically (solid lines). The intensity of experimental data points was normalized to the total integrated intensity of their respective spectra. **(C)** Schematic of femtosecond electron-probe scanning after the excitation of the Cu slab together with 3D field behavior (see text). **(D)** Energy level diagram in the kinematical and dynamical photon-electron exchange regimes.

strong- and weak-field regimes, respectively. The analogy to diffraction terminology reflects the number of particles involved; for our case here, it is a single-photon process, whereas in diffraction it is a single electron-scattering event (32). The strong-field limit can be seen in Fig. 3B, where seven EEGS and EELS peaks were observed for the Cu-vacuum interface. This is in contrast to the Ag nanoparticle case, where at most two orders were observed. Figure 4, A and B, shows the probe position dependence of several EEGS orders, experimentally and theoretically, together for comparison with the interface behavior of the Ag nanoparticle, which has a decay length of 55 nm into the vacuum. When the USI intensity of the first-order ($+1h\nu$) peak is plotted against the position of the probe, we clearly observe a buildup and decay with a peak 100 nm away from the interface. The theory outlined above predicts such behavior for the first and higher orders (solid lines in Fig. 4, A and B). Physically, this behavior is due to the

collective exchange of photons (see Fig. 4D) when the field is strong at the interface; 100 nm away, the field reaches the threshold intensity for the kinematical regime (i.e., the single photon-electron exchange). Thus, the buildup is due to the depletion of the $n = 1$ process with a concomitant rise in higher-order $n \neq 1$ processes until the threshold is reached at 100 nm. Another feature that is prominent for higher orders is the inclination in time-energy maps (Fig. 3B, dashed white line). This feature is a result of the chirp of electron pulses, but we do not analyze it in detail here.

Both the spectral and temporal resolutions in our setup are determined by the laser field, not the microscope-limited values of milliseconds and fractions of electron volts, respectively. Future developments may enable the nanometer-scale spatial resolution of our experiments to be extended to the atomic scale. High-order gain imaging can be exploited to further enhance the spatial localization and temporal response, as

demonstrated above. These combined dimensions of USI promise other applications in various domains, including those of molecules, particles, and possibly cells.

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become interactive, with an increase in the intensity for some peaks and a decrease in others, depending on the specimen and its thickness.

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Ohm's Law Survives to the Atomic Scale

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As silicon electronics approaches the atomic scale, interconnects and circuitry become comparable in size to the active device components. Maintaining low electrical resistivity at this scale is challenging because of the presence of confining surfaces and interfaces. We report on the fabrication of wires in silicon—only one atom tall and four atoms wide—with exceptionally low resistivity (~0.3 milliohm-centimeters) and the current-carrying capabilities of copper. By embedding phosphorus atoms within a silicon crystal with an average spacing of less than 1 nanometer, we achieved a diameter-independent resistivity, which demonstrates ohmic scaling to the atomic limit. Atomistic tight-binding calculations confirm the metallicity of these atomic-scale wires, which pave the way for single-atom device architectures for both classical and quantum information processing.

The continuous miniaturization (1–3) of classical as well as quantum electronic devices and circuitry for information processing relies on the implementation of low-resistivity leads and interconnects that are of the same scale as the active device components themselves (4, 5). At the fundamental limit of downscaling in semiconductor devices, functionality is obtained from only a few (6), or even single, dopant atoms (7–9) embedded within the semiconductor crystal. This emerging field of research, in which device properties are determined by a single dopant, is called solotronics (solitary dopant optoelectronics) (10). However, large-scale device architectures using multiple solitary dopants, such as donor-based quantum

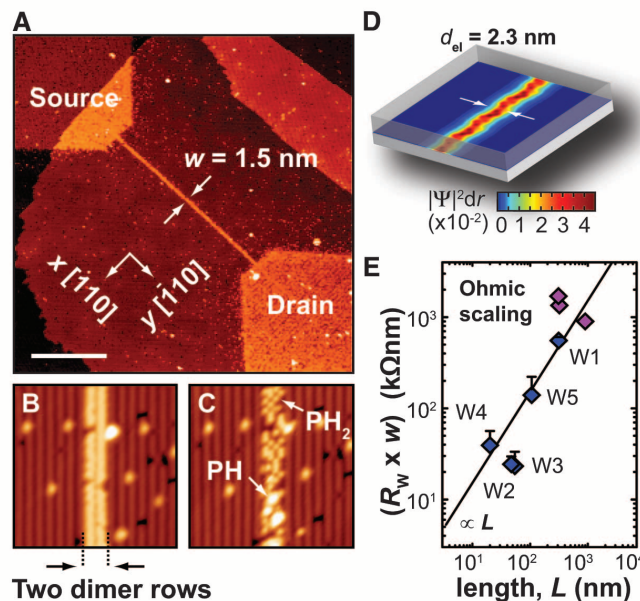
computers (11–14), require addressability of the individual dopants with interconnects comparable in size to the single-dopant Bohr radii ($a_B \sim$

2.5 nm for P in Si). In this size regime, maintaining low resistivity is challenging because of the increased ratio of surface or interface to volume that limits both the mobility and availability of free carriers. Indeed, in the sub-10-nm size regime, even heavily doped Si wires become highly resistive because of the limited doping density achievable (15) or as a result of the reduced doping efficiency (16) from the dielectric mismatch between the wire and its surroundings.

We demonstrate that bulk-like resistivities can be retained to the atomic scale by fabricating “interface-free” dopant wires embedded in single-crystalline Si. We used scanning probe lithography (6, 17) and a gaseous dopant source to write atomic-scale dopant wires on the Si(100) surface. We then embedded them within the Si bulk using molecular beam epitaxy (MBE), thereby removing them from surface and interface states. Figure 1A shows a scanning tunneling microscopy (STM) image of a 1.5-nm-wide wire template (corresponding to two dimer rows) extending

Fig. 1. Atomically abrupt dopant wires in silicon.

(A) STM image of a 4-atom-wide (1.5 nm), one-atom-tall, and 106-nm-long wire template, patterned along the <110> direction and connected to source/drain leads. Scale bar, 50 nm. (B and C) Atomic resolution images of a two-dimer-row-wide wire, before (B) and after (C) PH_3 dosing. After PH_3 exposure, the wire shows a large number of PH_x ($x = 1, 2$) fragments, strictly confined within the patterned regions. (D) Atomistic NEMO modeling of the electron distribution in a 1.5-nm dopant wire demonstrating tight charge confinement with a spread of the wave function outside the lithographic width. (E) Four-terminal resistances corrected for series resistances and normalized with the lithographic width ($R_w \times w$), plotted versus wire length L (blue and purple diamonds).



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to a length of 106 nm between the source and drain leads. The dark regions correspond to hydrogen-terminated Si(001), and the features with lighter contrast correspond to Si dangling bonds exposed by the STM-induced desorption of the hydrogen resist monolayer. Source and drain contact arms were also patterned by STM lithography in the same fabrication step, extending out to a total size of $\sim 2 \mu\text{m}$ (length) by $\sim 1 \mu\text{m}$ (width), which were used for ex situ alignment of metal contacts (17). Subsequent exposure to PH_3 gas selectively doped the wire template as well as the contact arms with a resolution set by the atomistic nature of the underlying reconstructed Si(001) surface. Atomic-resolution images of a two-dimer-row-wide template before and after dosing (Fig. 1, B and C) highlight the adsorption of PH_x ($x = 1, 2$) solely within the wire region. After a short anneal and epitaxial overgrowth, the P dopants were embedded in the silicon bulk

forming Si-P bonds, resulting in an atomically abrupt doping profile (18, 19) with extremely high planar doping density of ~ 0.25 monolayers ($N_D \sim 2 \times 10^{14} \text{ cm}^{-2}$) (20). In three dimensions (3D), this density corresponds to a value of $\sim 10^{21} \text{ cm}^{-3}$, three orders of magnitude beyond the Mott metal-insulator transition. The average dopant separation is $< 1 \text{ nm}$, smaller than the Bohr radius of a single donor ($a_B \sim 2.5 \text{ nm}$) (21). Consequently, the wires are crystalline, atomically abrupt, and expected to show metallic conduction.

The wires presented here are thin enough to allow a fully atomistic theoretical treatment using the tight-binding code NEMO-3D (22, 23). We calculated the self-consistent charge-potential profiles of the wires, taking into account both the high density and discrete nature of the doping. The wires were represented by an infinite repetition of a supercell in the $<110>$ direction, with the doping distribution matched to a doping den-

sity of 0.25 ML for each lithographic linewidth. The modeled wires were embedded within 70 nm of silicon in both transverse directions. The central 16 nm was modeled with an atomistic representation of the silicon lattice, while the remaining 54 nm was represented as a homogeneous dielectric with $\epsilon_{\text{Si}} = 11.7$. The density of states (DOS) was computed over the first Brillouin zone in 1D k -space, subject to charge neutrality in equilibrium, where the total electron number equals the number of positively ionized donors. Exchange and correlation effects are taken into account within the local density approximation (LDA) (24). The resultant electronic charge distribution along the thinnest wire (1.5 nm wide) is shown in Fig. 1D. The doping density changes by ~ 6 orders of magnitude from inside the wire ($N_D \sim 10^{21} \text{ cm}^{-3}$) to the lightly doped surrounding silicon substrate ($\sim 10^{15} \text{ cm}^{-3}$), giving an extremely small Thomas-Fermi screening length ($\sim 0.5 \text{ nm}$) and strong charge confinement.

Five different wires were measured (Table 1), all showing ohmic current-voltage (I - V) characteristics at $T = 4.2 \text{ K}$. At an applied bias of 500 μV (a low value was chosen to avoid electromigration damage), we achieved current densities of up to $5 \times 10^5 \text{ A cm}^{-2}$, which is near the maximum current densities of state-of-the-art copper interconnects ($\sim 10^6 \text{ A cm}^{-2}$) (5). To calculate the net wire resistance R_W , we first subtracted series resistances from the four-terminal resistances, which can be accurately determined by knowing both the sheet resistivity ($530 \Omega/\square$) and the exact geometry of each STM-patterned contact arm. Linear I - V characteristics with $R_W < 100 \text{ k}\Omega$ demonstrate the effective metallic doping down to a width of 1.5 nm. In Fig. 1E, we plot the wire resistance normalized by the lithographic width ($R_W \times w$) as a function of the wire length L . We include data from previously published results on wider STM-patterned wires (25) (purple diamonds) and observe ohmic scaling of the resistance for all devices, as typically observed in macroscopic metallic systems.

The unambiguous demonstration of ohmic scaling is a constant resistivity $\rho_W = R_W(A_{\text{el}}/L)$, independent of geometric variables such as wire length or width. When determining the resistivity of these wires, it is necessary to consider the effective electronic cross-sectional area, A_{el} , rather than their lithographic dimensions. Charge confinement is provided solely by the presence of the ionized dopants screened by both the silicon dielectric and the mobile electronic charge. As a consequence, the electronic width of the wire can extend into the surrounding silicon bulk and enlarge the effective diameter of the wire. The charge self-consistent NEMO-3D code captured this effect and allowed us to quantify A_{el} directly. Figure 2A shows the modeled radial charge distribution of the thinnest wire (W5), averaged over the length of the supercell. The decay of the charge distribution is given by $|\psi|^2 dr$, where $\psi \sim \exp(-r/a)$ represents the electronic wave function away from the core region of the doped wire

Table 1. Atomic dopant wires of different width. L and w denote the lithographic length and width of each wire studied. A_{el} and d_{el} are the effective electronic cross section and diameter of the wires as determined by atomistic calculations. Resistivities, ρ_W , were extracted from the wire resistance R_W using A_{el} . Measured wire resistances are compared to theoretical predictions R_{calc} based on the tight-binding calculations.

Sample	w (nm)	L (nm)	A_{el} (nm ²)	d_{el} (nm)	R_W (k Ω)	ρ_W (m $\Omega \text{ cm}$)	R_{calc} (k Ω)
W1	11.0	312	27.5	13.1	48.6	0.43	30 ± 4
W2	4.6	47	9.8	5.2	5.3	0.11	7 ± 1
W3	2.3	54	5.0	2.9	10.1	0.10	15 ± 2
W4	2.3	20	5.0	2.9	17.1	0.42	6 ± 1
W5	1.5	106	3.8	2.3	82.3	0.26	31 ± 4

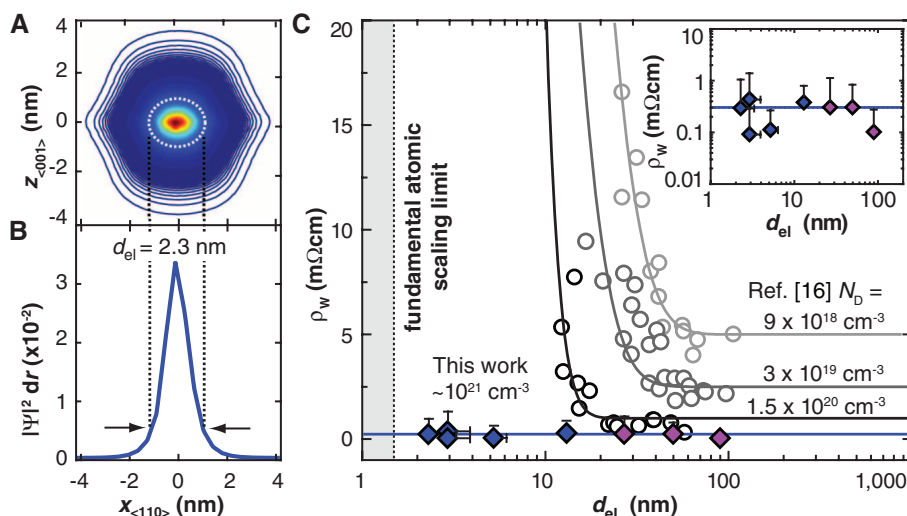


Fig. 2. Diameter-independent bulk-like resistivity down to the atomic limit. (A) Radial charge density $|\psi|^2 dr$ of the thinnest wire (W5) showing the effective cross-sectional area A_{el} (white dotted ellipse), used to calculate the wire resistivity ρ_W . (B) The corresponding effective wire diameter d_{el} (arrows) is determined from a cut through $|\psi|^2 dr$ in the plane of the dopants. (C) Resistivity $\rho_W(d_{\text{el}})$ of the STM-patterned wires (blue and purple diamonds), showing that it remains constant down to the fundamental scaling limit. The average value is near that for the silicon bulk resistivity at equivalent doping density (blue line). This is in contrast to an exponential deviation from bulk values as found in VLS-grown nanowires. [Open circles, data graphically extracted from (16). The lines are guides to the eye.] (Inset) $\rho_W(d_{\text{el}})$ on a double-logarithmic scale.

with a decay length a defined analogously to the Bohr radius a_B of an isolated P donor. By considering a in all radial directions, we quantify A_{el} , where ψ decays to $1/e$ of its peak value, as shown by the dotted line in Fig. 2A. Thus, $>70\%$ of the total electronic charge is enclosed in the modeling domain. In turn, this also allowed us to determine the effective electronic diameter d_{el} of the wire (arrows in Fig. 2B). For W5, we found $d_{el} = 2.3$ nm compared with $w = 1.5$ nm (see Table 1). We simulated several supercell configurations with different dopant positioning within the wire, which revealed negligible impact on either A_{el} or d_{el} down to the thinnest wire.

In Fig. 2C, we compare the resistivity ρ_W as a function of the wire diameter d_{el} (blue and purple diamonds) with reported values for other silicon wires. It is evident that for the STM-patterned wires, ρ_W remains constant despite d_{el} varying over nearly two orders of magnitude. The inset shows ρ_W on a double-logarithmic scale, revealing a minimum resistivity of 0.1 m Ω cm (W3), the lowest reported in doped silicon wires. The average resistivity ($\rho_W = 0.3 \pm 0.2$ m Ω cm) is comparable to that of bulk-doped silicon of similar doping density (26) (blue line). The relatively large spread of resistivity at such low values can arise from sample-to-sample variations in the spatial distribution of the dopants within the wires. Our data contrasts sharply with the behavior of other doped Si wires previously reported, where strong deviations from bulk values of the resistivity were typically observed below $d \sim 10$ nm (15, 16). We have reproduced the resistivity data of vapor-liquid-solid (VLS)-grown wires with three different doping densities, measured by Björk *et al.* (16), where the resistivity is observed to increase exponentially with decreasing diameter. This resistivity increase was more pronounced for the lower doping densities and prevailed even when the wire and resistivities were corrected for a depletion width arising from surface and interface states. Björk *et al.* attribute this exponential rise to suppression of dopant activation and a concomitant decrease in free-carrier density. Theoretical calculations by Diarra *et al.* (27) predict a d^{-1} dependence of dopant ionization energy for $d < 10$ nm, induced by a mismatch in the dielectric constants of the silicon wire (ϵ_{in}) and its environment (ϵ_{out}). For $\epsilon_{in} > \epsilon_{out}$, the dopant core potential is insufficiently screened, resulting in an increase in ionization energy. The exponential divergence persists to the highest doping density achievable in VLS growth (15), indicating the existence of a scaling barrier at $d \sim 10$ nm for doped silicon wires.

In contrast, by embedding the doped wires within bulk silicon, we eliminated the dielectric mismatch ($\epsilon_{in} = \epsilon_{out}$) and thereby achieved full dopant activation. This feature, combined with atomically doping the silicon wire to extremely high densities using a gaseous dopant source, allowed us to overcome the apparent scaling barrier and resulted in the persistence of bulk-like resistivity down to $d_{el} = 2.3$ nm ($w = 1.5$ nm).

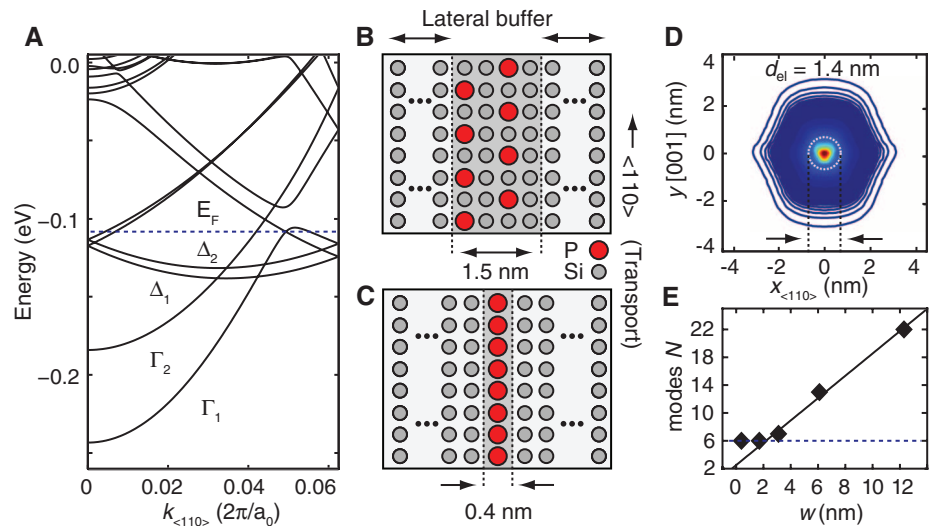


Fig. 3. Atomic-scale dopant wires at the scaling limit. **(A)** Band structure of the 1.5-nm-wide wire (W5) displaying band metallicity with six propagating modes ($N = 6$) at $E_F = 135$ meV, measured from the Γ_1 minimum. **(B and C)** Supercell configuration for the 1.5-nm-wide wire (B), as well as a single atomic chain of dopants (C) used to calculate the charge-potential profiles and band structures. **(D)** Radial charge distribution of a single atomic chain, representing the fundamental scaling limit for dopant wires in silicon. While maintaining $N = 6$, the electronic diameter $d_{el} = 1.4$ nm represents the fundamental scaling limit for dopant wires in silicon. **(E)** The number of modes N at the Fermi energy as a function of wire width w used to calculate wire resistances R_{calc} .

One of the advantages of atomistic tight-binding modeling is that we can determine the number of propagating metallic modes at the Fermi energy for different wire widths and from this estimate the resistance of the narrowest conducting STM-patterned doped wires in silicon. Figure 3A shows the self-consistent band structure calculation for the narrowest STM-patterned wire W5 (1.5 nm) based on the supercell configuration shown in Fig. 3B. We observed strong band bending, so that the minimum of the lowest occupied band (Γ_1) lies 243 meV below the conduction band edge of bulk silicon ($E = 0$). The Fermi energy E_F , indicated by the blue dotted lines, is situated well above the Γ_1 minimum (135 meV), making the wire a band-metal at $T = 4.2$ K. For this heavily doped silicon system, we observed occupation of the lowest sub-band in all six valleys of the Si conduction band, whose degeneracy is lifted by the strong vertical and lateral quantum confinement of the wires, giving $N = 6$ propagating modes at E_F (6, 21).

We determined the number of modes in these doped silicon wires as we dropped the lithographic width down to an ideal single chain of individual dopants, represented by the supercell configuration in Fig. 3C. Below $w \sim 2$ nm, we observed a saturation of the number of propagating modes at E_F ($N = 6$), again reflecting the six conduction band valleys in silicon (Fig. 3E). The knowledge of $N(w)$, along with estimates of the electron mean free path in Si:P δ -doped systems ($l = 8 \pm 1$ nm) (28), allowed us to calculate the resistance $R_{calc} = (h/2e^2N)(1 + L/l)$ in the diffusive regime of a multimode quasi-1D metal, where ($L \gg l$) (29). We obtained good agreement with resist-

ances obtained experimentally for all wires, as shown in Table 1. Small deviations could be explained by sample-to-sample fluctuations of the conductance (30), expected in the diffusive regime and the potential onset of electron localization at low temperature (29) not captured in this model. We also determined the absolute physical limit to which doped silicon wires could be scaled and found the radial charge distribution of a single dopant chain to be ~ 1.4 nm (Fig. 3D). This number therefore defines the fundamental atomic scaling limit (vertical line in Fig. 2C) of doped silicon wires.

These STM-patterned wires achieve charge confinement in the absence of any surface or material interface. The combination of extremely high doping density and atomically abrupt dopant positioning in a crystalline environment provides an unprecedented scalability to atomic-scale dimensions, yet retains a diameter-independent, bulk-like resistivity. The resulting persistence of Ohm's law at the atomic limit paves the way for ultrascaled classical as well as quantum electronic components, such as source-drain leads, interconnects, and local electrostatic gates necessary to electrically address individual dopants in solitronic and donor-based quantum computing architectures.

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Candle Soot as a Template for a Transparent Robust Superamphiphobic Coating

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Coating is an essential step in adjusting the surface properties of materials. Superhydrophobic coatings with contact angles greater than 150° and roll-off angles below 10° for water have been developed, based on low-energy surfaces and roughness on the nano- and micrometer scales. However, these surfaces are still wetted by organic liquids such as surfactant-based solutions, alcohols, or alkanes. Coatings that are simultaneously superhydrophobic and superoleophobic are rare. We designed an easily fabricated, transparent, and oil-rebounding superamphiphobic coating. A porous deposit of candle soot was coated with a 25-nanometer-thick silica shell. The black coating became transparent after calcination at 600°C. After silanization, the coating was superamphiphobic and remained so even after its top layer was damaged by sand impingement.

A major goal in coating research is to design self-cleaning surfaces (1–4). Many surfaces in nature are superhydrophobic; for example, lotus leaves (5). Mimicking their surface morphology led to the development of a number of artificial superhydrophobic surfaces (6, 7), opening many applications in industrial and biological processes (8–13). Microscopic pockets of air are trapped beneath the water drops (14–17). This composite interface leads to an increase in the macroscopic contact angle and a reduced contact angle hysteresis, enabling water drops to roll off easily, taking dirt with them. However, the addition of an organic liquid such as alcohol or oil decreases the interfacial tension sufficiently to induce homogeneous wetting of the surface. Drops, initially resting on air pockets (in a Cassie state), pass the transition to com-

plete wetting (a Wenzel state) (14). No naturally occurring surface is known to show a contact angle θ greater than 150° and roll-off angles below 10° for water and organic liquids. These superhydrophobic and superoleophobic surfaces are called superamphiphobic (18).

In contrast to superhydrophobicity, the term “superamphiphobicity” is not uniquely defined, in particular with respect to the liquid used (19–22). According to Young’s equation, $\cos\Theta = (\gamma_{SV} - \gamma_{SL})/\gamma_{LV}$, the lower the surface tension, the higher the tendency of a liquid to spread on a solid surface (22, 23). Here, Θ is the macroscopic contact angle, γ_{SV} is the surface tension of the solid, and γ_{SL} is the interfacial tension of the solid/liquid interface. For organic liquids ($30 \leq \gamma_{LV} \leq 18$ mN/m), mainly van der Waals interactions act between the molecules. Therefore, $\gamma_{SV} - \gamma_{SL}$ is positive, and on planar surfaces $\Theta < 90^\circ$. Similarly, the contact angle on rough surfaces depends on the surface tensions, because roughness amplifies the wetting properties.

The key factors for superamphiphobicity are not clear yet. For water repellency, surface rough-

ness and low surface energy are essential (14). To fabricate superamphiphobic surface overhangs, reentrant geometry or convex curvature is also important (19–25). The complex interplay between surface roughness, low surface energy, and topography has made it difficult and expensive to fabricate superamphiphobic surfaces. Tuteja *et al.* showed that careful design of the topography of a surface allows the construction of surfaces with a contact angle for hexadecane close to 160°, although the flat surface was oleophilic (19, 23). They explained their exceptional oil-repellency by overhang structures and reentrant geometry.

Here, we describe a simple way to make robust, transparent, superamphiphobic coatings. The surface to be coated, in our case a glass slide, is held above the flame of a paraffin candle (Fig. 1A). Deposition of a soot layer turns the glass black. Scanning electron microscopy reveals that the soot consists of carbon particles with a typical diameter of 30 to 40 nm, forming a loose, fractal-like network (Fig. 1, B and C) (26). A water drop gently deposited on the surface shows a contact angle above 160° and rolls off easily, demonstrating the surface’s superhydrophobicity (27). However, the structure is fragile because the particle-particle interactions are only physical and are weak. When water rolls off the surface, the drop carries soot particles with it until almost all of the soot deposit is removed and the drop undergoes a wetting transition (movie S1).

Inspired by the promising morphology of soot, we developed a technique to coat the soot layer with a silica shell, making use of chemical vapor deposition (CVD) of tetraethoxysilane (TES) catalyzed by ammonia. The soot-coated substrates were placed in a desiccator together with two open glass vessels containing TES and ammonia, respectively (fig. S1). Similar to a Stöber reaction, silica is formed by hydrolysis and condensation of TES. The shell thickness can be tuned by the duration of CVD. After 24 hours, the particles were coated by a 20 ± 5 -nm-thick silica shell (Fig. 1, D and E). Calcinating the

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hybrid carbon/silica network at 600°C for 2 hours in air caused combustion of the carbon core (Fig. 1F) and a decrease in the shell thickness, but the layer kept its roughness and network texture. Only isolated chains of particles, which were not linked in the network, broke during calcination (Fig. 1B). To reduce the surface energy, the hydrophilic silica shells were coated with a semi-fluorinated silane by CVD. Therefore, the substrates and an open beaker with the volatile silane were put in a desiccator for 3 hours. After CVD, a water drop placed on top of the coating formed a static contact angle of $165^\circ \pm 1^\circ$ (Fig. 2A), with a roll-off angle lower than 1° . Owing to the extremely low adhesion of the coating to water, it was difficult to deposit water drops, because they immediately rolled off (movie S2). When drops of organic liquid were deposited, the static contact angles ranged from 154° for tetradecane up to 162° for diiodomethane (Fig. 2B, Table 1, and fig. S3). The maximal roll-off angle was 5° , even for tetradecane with a surface tension of 26 mN/m.

Hexadecane drops with a radius of 1 mm, impinging with a velocity up to $v = 1$ m/s, did not penetrate into the layer. The drop's kinetic energy was transformed into vibrational energy, allowing the drop to rebound twice before it underwent damped oscillations and finally rested on the surface in the Cassie state (Fig. 2D, figs. S4 and S5, and table S1) (28–30). The coating retained its superamphiphobicity even after the impingement of at least thousands of water drops with a radius of 1.3 mm and a velocity of 1.4 m/s (fig. S6) or flushing of the coating with water for several hours.

At velocities between 1 and 1.5 m/s, the drop started to penetrate into the coating. As a result, a satellite drop was left on the surface after rebound. Typically at the second impact, the satellite drop merged with the primary drop and rolled off (fig. S5). Self-cleaning properties for water and alkane were verified by depositing drops of either liquid on a superamphiphobic layer and monitoring the taking up of contaminants (fig. S7).

For applications on glass surfaces such as goggles, touch screens, or difficult-to-access windows, the superamphiphobic coating needs to be thermally stable, transparent, and mechanically robust. To quantify the thermal stability, the coatings were annealed at temperatures up to 450°C for 1 hour. The static contact and roll-off angles remained constant up to 400°C (Fig. 3A). Annealing at even higher temperatures decomposed the fluorosilane. The silica network remained almost unaltered until annealed at temperatures up to 1000°C (fig. S8). Annealed coatings can recover their superamphiphobicity after repeating CVD of a fluorosilane. After calcination of the black carbon template, the silica network has a shell thickness well below the wavelength of light. Such thin shells are highly transparent, as verified by ultraviolet-visible transmittance spectra (Fig. 3B). The transmittance of a 3- μ m-thick

Fig. 1. Morphology of porous structure. (A) Photograph depicting sample preparation. A glass slide is held in the flame of a candle until a soot layer a few micrometers thick is deposited. (B) Scanning electron microscope (SEM) image of the soot deposit. (C) High-resolution SEM image showing a single particle chain made up of almost spherical carbon beads 40 ± 10 nm in diameter. (D) SEM image of the deposit after being coated with a silica shell (see fig. S2 for a cross section of the deposit). (E) High-resolution SEM image of a cluster after the carbon core was removed by heating for 2 hours at 600°C. (F) High-resolution TEM image of a cluster after calcination, revealing the silica coating with holes that were previously filled with carbon particles. The silica shell is 20 ± 5 nm thick.

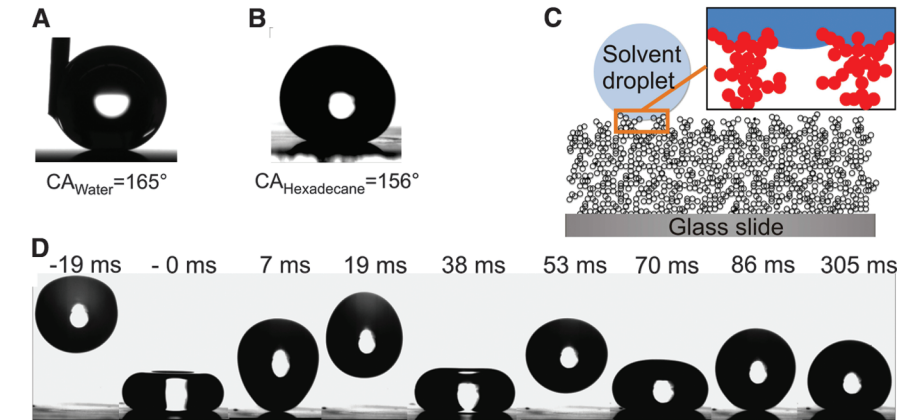
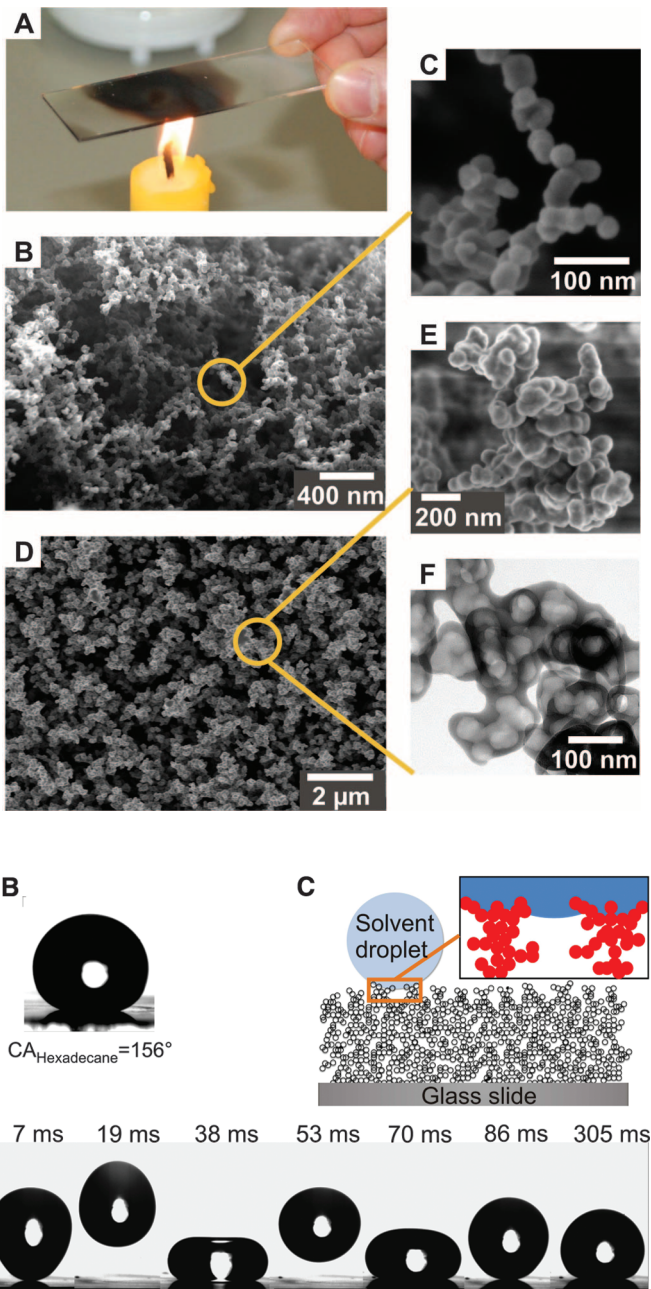


Fig. 2. Superamphiphobicity of the surface. A 2- μ l water drop (A) and 5- μ l hexadecane drop (B) deposited on the surface possess a static contact angle of $165^\circ \pm 1^\circ$ and $156^\circ \pm 1^\circ$, respectively. (C) Cartoon of a liquid drop deposited on the fractal-like composite interface. (D) Time-resolved images of the bouncing of a 5- μ l hexadecane drop on a superamphiphobic surface. Just before impinging, the drop's kinetic energy exceeds its interfacial energy by 2.4 (that is, the Weber number is 2.4) (28).

Table 1. Comparison of the static contact angle (SCA) and roll-off angle of drops with different surface tension, deposited on a flat fluorinated glass substrate and on a superamphiphobic coating.

Liquid	Surface tension (mN/m)	Flat surface SCA°	Superamphiphobic surface SCA°	Roll-off angle°
Water	72.1	108 ± 1	165 ± 1	1 ± 1
Diiodomethane	50.9	91 ± 1	161 ± 1	2 ± 1
Ethylene glycol	47.3	89 ± 1	160 ± 1	2 ± 1
Peanut oil	34.5	70 ± 1	158 ± 1	4 ± 1
Olive oil	32.0	69 ± 1	157 ± 1	4 ± 1
Hexadecane	27.5	64 ± 1	156 ± 1	5 ± 1
Tetradecane	26.5	54 ± 1	154 ± 1	5 ± 1

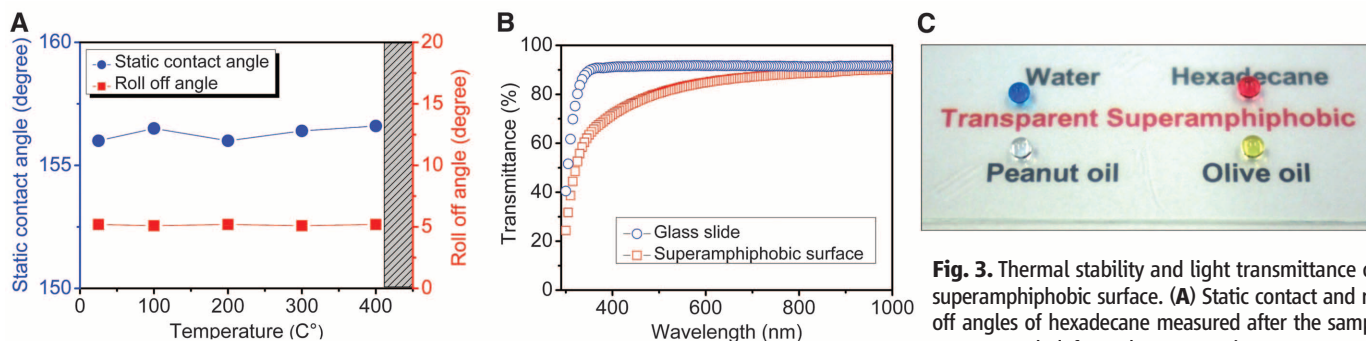


Fig. 3. Thermal stability and light transmittance of a superamphiphobic surface. **(A)** Static contact and roll-off angles of hexadecane measured after the samples were annealed for 1 hour at various temperatures. **(B)** Ultraviolet-visible transmittance spectra of a 3- μm -thick superamphiphobic surface compared to pristine glass. **(C)** Photograph of a drop of dyed water ($\gamma_{\text{lv}} = 72.1$ mN/m, blue); peanut oil ($\gamma_{\text{lv}} = 34.5$ mN/m, white); olive oil ($\gamma_{\text{lv}} = 32.0$ mN/m, yellow); and dyed hexadecane ($\gamma_{\text{lv}} = 27.5$ mN/m, red) deposited on a superamphiphobic glass slide. The coated slide was placed on labeled paper.

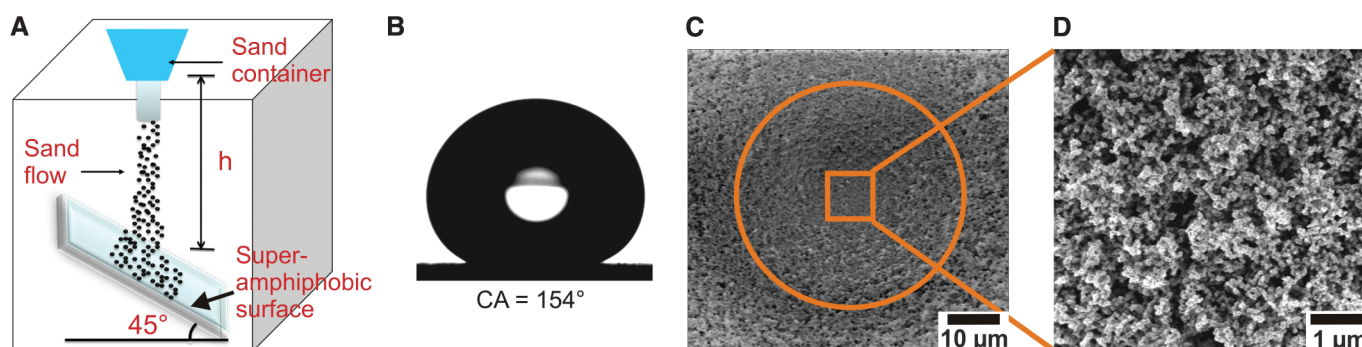


Fig. 4. Mechanical resistance quantified by sand abrasion. **(A)** Schematic drawing of a sand abrasion experiment. **(B)** Hexadecane drop deposited on the coating after 20 g of sand abrasion from 40 cm height. The 100- to 300- μm -sized grains had a velocity of 11 km/hour just before

impingement. After impingement, the drops rolled off after the substrate was tilted by 5°. **(C)** SEM image of a spherical crater (orange circle) after sand abrasion. **(D)** SEM image of the surface topography inside the cavity.

coating is reduced by less than 10% as compared to that of pristine glass for wavelengths above 500 nm. This transparency is reflected in the easy readability of letters underneath the coated glass plate, and its superamphiphobicity is shown by the high contact angle for a wide variety of liquid drops (Fig. 3C).

In outdoor applications, superamphiphobic surfaces need to survive harsh conditions. To investigate the mechanical resistance of our coating, water-drop impact and sand abrasion tests were performed. Sand grains 100 to 300 μm in diameter impinged the surface from a height of 10 to ~40 cm, corresponding to an impinging energy of 1×10^{-8} to 90×10^{-8} J per grain (Fig. 4A). The silica shells were not sufficiently robust to completely resist sand impact. A cave formed underneath the impacted area (Fig. 4C). However, zooming into the cave revealed an almost unaltered submicrometer morphology (Fig. 4D). Owing to the coating's self-similarity, the surface kept its superamphiphobicity until the layer was removed after extended impact. The mechanical durability depended on the amount of sand impinging per unit of time and area, the size of the grains, the height of fall, and the thickness of the silica shell. The mechanical stability

increased with the thickness of the silica shell, but at the expense of the coating's transparency. The surface retained its superamphiphobicity for 5 min of sand abrasion from a height of 25 cm (2 m/s) (movie S3). Although the coating can be eroded by wear and abrasion, it keeps its superamphiphobicity as long as its thickness remains above 2 μm (fig. S11).

The coating consists of a fractal-like assembly of nanospheres. With increasing duration of CVD of TES or annealing above 1100°C, the necks between particles fill with silica and more rod-like shapes evolve, which reduces the superamphiphobicity (figs. S8 and S10). This can be understood from Nosonovsky's prediction that convex small-scale roughness can provide a sufficient energy barrier against wetting (22, 31), thus rendering superamphiphobicity possible. A spherical shape should provide a higher-energy barrier against wetting than a rod-like shape (figs. S8 and S10).

Our easy-to-fabricate oil- and water-repellent coating is made from soot encased in a silica shell. The coating is sufficiently oil-repellent to cause the rebound of impacting drops of hexadecane. Even low-surface-tension drops of tetradecane roll off easily when the surface is tilted

by 5°, taking impurities along with them. The surface keeps its superamphiphobicity after being annealed at 400°C. The coating is transparent and can be applied to a variety of heat-resistant surfaces, such as aluminum, copper, or stainless steel.

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Supporting Online Material

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Movies S1 to S3

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Capturing Ultrasmall EMT Zeolite from Template-Free Systems

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Small differences between the lattice energies of different zeolites suggest that kinetic factors are of major importance in controlling zeolite nucleation. Thus, it is critical to control the nucleation kinetics in order to obtain a desired microporous material. Here, we demonstrate how careful investigation of the very early stages of zeolite crystallization in colloidal systems can provide access to important nanoscale zeolite phases while avoiding the use of expensive organic templates. We report the effective synthesis of ultrasmall (6- to 15-nanometer) crystals of the large-pore zeolite EMT from template-free colloidal precursors at low temperature (30°C) and very high yield.

Zeolites are metastable crystalline aluminosilicate molecular sieves with uniform pores of molecular dimensions that are widely applied in catalysis, separations, and adsorption (1–4). The EMT-type zeolite has one of the lowest framework densities for a microporous material (5) and is a hexagonal polytype of the cubic FAU-type zeolite that plays a very important role in catalysis, for example, in fluid catalytic cracking (FCC) of hydrocarbons (6). Similar to the FAU-type material, the EMT framework topology has a three-dimensional large (12-membered ring) pore system. The cubic FAU polymorph features only one type of supercage (with a volume of 1.15 nm³), but a different stacking of faujasite sheets creates two cages in the EMT zeolite: a hypocage (0.61 nm³) and a hypercage (1.24 nm³) (7). The EMT material shows interesting catalytic properties different from FAU as an FCC catalyst, but the very high price of the product so far precludes practical uses (8, 9).

In addition, several EMT-FAU intergrown phases (CSZ-1, ECR-30, ZSM-20, ZSM-3) have also been reported (9–14). The synthesis of pure EMT-type zeolite is possible by templating with the expensive 18-crown-6 ether and using tightly

controlled synthesis parameters (7). Many studies have been carried out to reduce the consumption of the crown ether template, for instance, by recycling after the synthesis (15) or using the so-called “SINTEF” tumbling approach (16, 17), steam-assisted crystallization (18), surfactants (19), or other organic and inorganic auxiliary additives (20–22). Although the cost of producing EMT has been reduced, all attempts toward a synthesis of EMT-type zeolite without an organic structure-directing agent (OSDA) have been un-

successful thus far. Moreover, the 18-crown-6 ether template stimulates the crystallization of micrometer-sized EMT crystals, and no attempts for the preparation of nanosized crystals have been reported.

Certain nanosized molecular sieves have been obtained at moderate (60° to 130°C) and low temperatures (25° to 50°C) (23–26). Low-temperature synthesis techniques for discrete zeolite nanocrystals from organic-free precursor systems are highly desired, as they would reduce cost and hazardous wastes, save energy, and possibly alter the properties of the materials (26, 27). Here, we describe a template-free Na₂O–Al₂O₃–SiO₂–H₂O precursor system as a foundation for the preparation of a nanosized EMT molecular sieve, where the ratios between different compounds, nucleation temperature and times, and type of heating have been adjusted to avoid phase transformations (e.g., to FAU and SOD) and to stabilize the EMT-type crystals at a small particle size. We report the synthesis of ultrasmall hexagonal EMT nanocrystals (diameter of 6 to 15 nm) at the low temperature of 30°C without using any organic template; that is, from Na-rich precursor suspensions. Strikingly, this synthesis strategy requires no organic

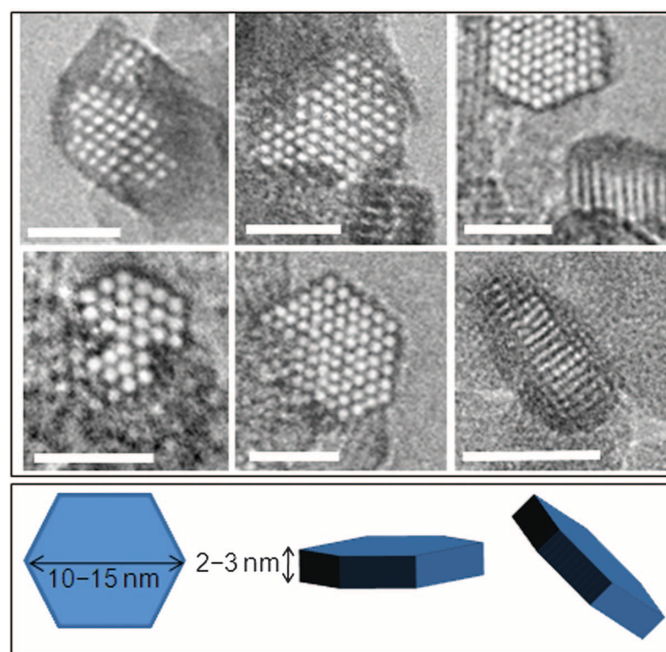


Fig. 1. Ultrasmall EMT crystals with hexagonal morphology synthesized from template-free precursor suspension at 30°C for 36 hours. The individual crystals are schematically presented with a size of 10 to 15 nm and a thickness of 2 to 3 nm. Scale bars, 10 nm.

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Fig. 2. XRD patterns representing the evolution of ultrasmall EMT crystals from template-free precursor suspensions at 30°C under conventional heating for (A) 8 hours, (B) 14 hours, (C) 24 hours, and (D) 36 hours. Vertical ticks correspond to line indexing of the EMT phase. The difference diagram between calculated and experimental points is shown at the bottom of each XRD pattern. (Insets) Crystallite sizes and shapes calculated based on the XRD data. The whole pattern was fitted using the combined analysis formalism (30) implemented in the MAUD program (31), based on the Rietveld analysis approach. Fourier analysis was applied to deconvolute the instrumental- and sample-broadening parts from the measured XRD lines. The instrumental contribution to the line broadening was calibrated with an LaB_6 standard powder from the National Institute of Standards and Technology. The Popa formalism was then used to describe anisotropic crystallite sizes and shapes (32), and an arbitrary texture correction model (31) was used to account for the moderate preferred orientations introduced in the EMT powder in a flat sample holder. a.u., arbitrary units.

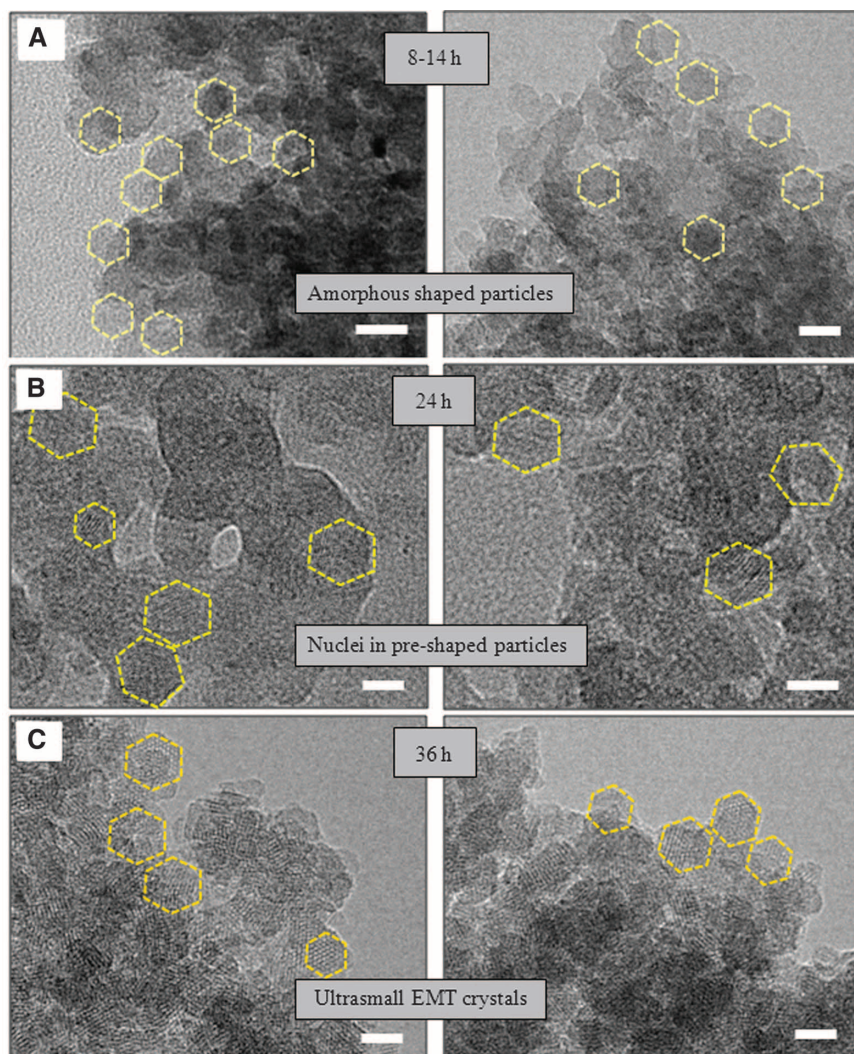
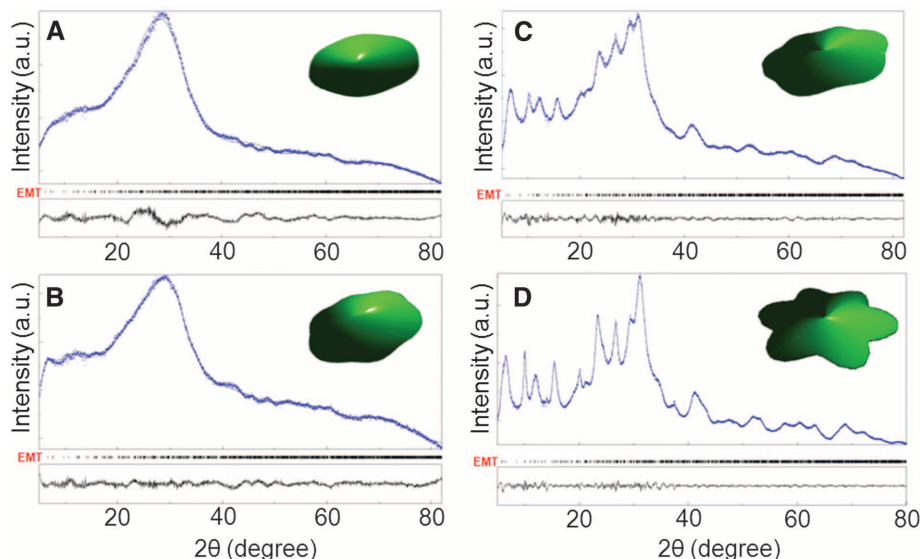


Fig. 3. TEM images of (A) amorphous-shaped particles in the template-free precursor suspension after 8 to 14 hours, (B) birth of ultrasmall EMT nuclei after 24 hours, and (C) fully crystalline ultrasmall EMT after 36 hours of conventional heating at 30°C. Scale bars, 10 nm.

template. Moreover, the absence of an organic template implies that no high-temperature calcination step is required for opening up the pore system for the intended applications.

Typically, a high concentration of OSDA is needed to prepare nanosized zeolites to achieve a high degree of supersaturation by which the crystal size can be controlled and the resulting nanoparticles can be stabilized. We synthesized the ultrasmall (6- to 15-nm) and nanosized (50- to 70-nm) EMT crystals at near ambient conditions within a very short time; that is, 36 hours under conventional heating and 4 min under microwave irradiation, respectively (see supporting online material, fig. S1). We used these “soft” conditions to avoid the formation of the more stable and denser phase hydroxysodalite (SOD) and the formation of a FAU-type phase observed in precursor suspensions with slight changes of the oxide ratios and water content (fig. S2).

The ultrasmall EMT crystals prepared at 30°C for 36 hours under conventional heating are shown in Fig. 1. Nearly 63% of the amorphous aluminosilicates were transformed into ultrasmall hexagonal EMT zeolite ($\text{Si}/\text{Al} = 1.14$). The particles were single crystals with a size of ~6 to 15 nm, and they contained channels in a highly ordered hexagonal arrangement. The x-ray diffraction (XRD) pattern of this sample (fully crystalline EMT-type zeolite) exhibited broadened Bragg peaks, which suggests the presence of very small crystallite sizes (Fig. 2D). The pattern was indexed using the hexagonal EMT structure in the $\text{P6}_3/\text{mmc}$ space group, with a reliability factor as low as weighted $R_w = 1.5\%$, Bragg $R_B = 1.12\%$, and experimental $R_{\text{exp}} = 0.62\%$, which gave rise to a goodness-of-fit of 6. In addition, zeolites X and Y (FAU-type structures) were considered in the modeling; however, they did not fit the first three peaks characteristic of the EMT zeolite, nor the anisotropic line broadening (fig. S3).

The refined cell parameters, $a = 1.7616(1)$ nm and $c = 2.838(2)$ nm, correspond very well to the EMT-type structure (5). Moreover, the refined shape of the particles was a hexagonal crystal with mean sizes of 10 nm along the [100] direction, 15 nm along [110], and 2.0 nm along [001]. The refined shape of the crystallites matched the size and shape of the EMT crystals measured with high-resolution transmission electron microscopy (HRTEM) (Figs. 1 and 2D). Moreover, the fitting of this XRD pattern (sample synthesized at 30°C for 36 hours) with isotropic and platelike EMT crystals did not provide better fitting results (fig. S3 and table S1).

We investigated the entire process of nucleation and growth of the ultrasmall EMT crystals in the system subjected to conventional heating for a total period of 36 hours. HRTEM images of the solid particles extracted at different crystallization times revealed the presence of amorphous gel after 8 to 14 hours and fully crystalline EMT particles after 36 hours of heating at 30°C (Fig. 3). We did not observe any difference between the TEM pictures of samples heated for 8 and 14 hours. Amorphous objects of about equal size with a diameter of 2 to 10 nm were seen in these samples (Fig. 3A); some of these particles had a size and morphology similar to the final EMT crystallites. The XRD pattern of the sample heated only for 8 hours did not exhibit any Bragg peaks, confirming the amorphous nature (Fig. 2A), whereas analysis of the diffraction pattern for the sample heated for 14 hours (Fig. 2B) resulted in nonsatisfactory fitting based on the use of one phase only (either amorphous or crystalline). Thus, we used a mixture of amorphous and

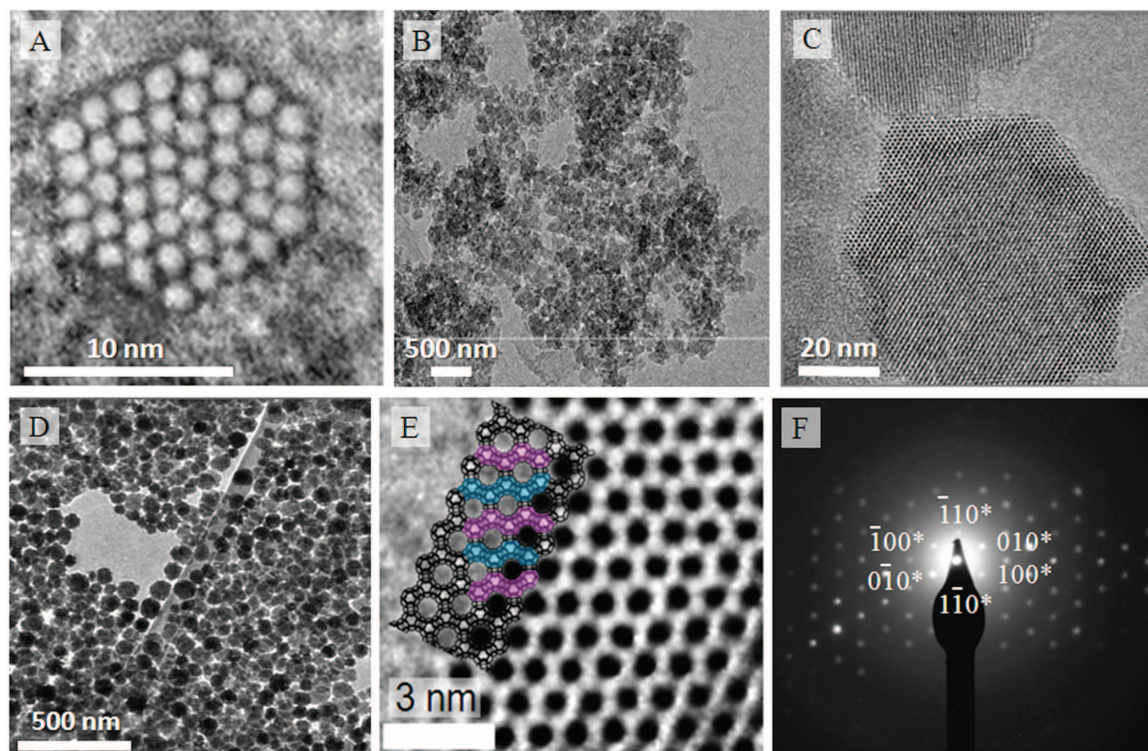
nanocrystalline EMT-type zeolite for the fitting. On the basis of this refinement (Fig. 2B), the relative amount of the crystalline phase calculated is ~30%. More developed anisotropy of both unit-cell ($c/a = 1.44$) and mean crystallite shape ($2.1 \times 2.3 \times 1.0$ nm³) was calculated for this sample based on the XRD. Although the XRD pattern corresponds to an entirely amorphous sample, based on the Rietveld refinement, we concluded that the particles had anisotropic shapes with mostly developed hexagonal platelike morphology (fig. S4 and table S2) (28). Moreover, the refined crystallite volume was comparable to the unit-cell volume, which is a signature of the starting of EMT growth (fig. S5). After 24 hours of heating, ultrasmall crystallites of zeolite EMT appeared in the amorphous matrix (Fig. 3B). The XRD pattern of this sample contains amorphous matter and low intense Bragg peaks (Fig. 2C). The size of the crystalline domains calculated based on XRD is $5.5 \times 2.0 \times 5.1$ nm³ (table S3). Moreover, the crystalline particles existing in the aluminosilicate system after 14 and 24 hours heating at 30°C already exhibited the hexagonal shape (insets in Fig. 2, B and C).

As the crystallization proceeded, the intensity of the Bragg peaks increased, but they were still broad because of the small crystalline domains. After 36 hours of heating, the amorphous particles were turned entirely into crystalline matter, as demonstrated with HRTEM and XRD (Figs. 2D and 3C). In the fully crystalline sample, we detected well-formed hexagonal particles with crystalline fringes. A closer look at these nanocrystals (Fig. 1) revealed that the apparent size and the hexagonal arrangement of the micropores corre-

spond to the EMT-type zeolite. Further evidence for the high crystallinity of the samples was provided by N₂ sorption and spectroscopic data (infrared and nuclear magnetic resonance spectroscopy) (figs. S6 to S9 and table S4). The nitrogen sorption measurement of the fully crystalline sample (36 hours) revealed a type I sorption isotherm; micropores of 7.3 Å were observed in addition to textural mesoporosity (pores of 2 to 50 nm) attributed to the random packing of the ultrasmall nanocrystals. The Brunauer-Emmett-Teller surface area for the ultrasmall and nanosized EMT materials was 578 and 562 m²/g, and the pore volume was 0.78 and 0.84 cm³/g, respectively (table S4).

We compared the ultrasmall EMT crystals synthesized under conventional heating (Fig. 4, A and B) with nanosized EMT crystals synthesized under microwave heating (Fig. 4, C and D) at 30°C. The HRTEM images of the sample prepared with microwave treatment revealed the presence of small EMT crystals with the characteristic hexagonal platelike morphology (Fig. 4C). The crystalline solids showed a uniform particle size of 50 to 70 nm (Fig. 4D and figs. S1 and S10). The selected-area electron diffraction (SAED) pattern of the nanosized EMT exhibits sixfold symmetry, which is indicative of the EMT structural features (Fig. 4F and table S5). The distance between two fringes based on the TEM measurement is 1.3 nm. The ABABAB stacking of the faujasite sheets is observed in the EMT nanocrystals; this stacking gives the hexagonal crystal shape and EMT topology (Fig. 4E). Unlike high silica micrometer-sized EMT zeolite, the nanosized EMT grow favorably in the a direction, rather

Fig. 4. TEM images of ultrasmall EMT crystals at different magnifications with scale bars of (A) 10 nm and (B) 500 nm, as well as nanosized EMT single crystals with scale bars of (C) 20 nm and (D) 500 nm. (E) Magnified TEM image and corresponding schematic diagram of the framework structure (ABABA stacking of faujasite sheets highlighted in purple and blue). (F) SAED pattern of a nanosized EMT single crystal, projected along [100].



than in the *c* direction, thus resulting in the thin hexagonal-plate form due to the limited crystal growth via the layer-by-layer mechanism along the *c* direction (29).

The homogeneity of the two samples, ultra-small (6- to 15-nm) and nanosized (50- to 70 nm) EMT crystals, is illustrated at two different magnifications in Fig. 4, A to D. The hexagonal morphology of both samples is evident. Moreover, their colloidal stability was examined by measuring the zeta potential values, which are equal to -45 mV. This negative charge leads to electrostatic stabilization of the hexagonal nanoparticles.

Why has EMT never been observed in organic-free synthesis solutions? The reason may be surprisingly simple. When the same synthesis solutions as described above were heated at the same temperature for extended times or at higher temperatures, the nanoscale EMT materials converted into the well-known FAU and SOD structures (fig. S2). We propose that under appropriate conditions the EMT is the first kinetic, metastable product in this synthesis field, followed by conversion into the more stable cubic FAU and more dense SOD structures. This hypothesis is strongly supported by several reports on EMT/FAU intergrowths (9–14). Indeed, we suggest that it may be possible to capture other important zeolite phases that occur as intergrowths by exploiting the very early stages of synthesis and thus avoiding the use of organic reagents that are commonly needed to stabilize the desired phases.

From an environmental perspective, the synthesis of EMT zeolite presented here is extremely attractive, as the nanocrystals can be easily synthesized at very high yield at near ambient temperature without using any organic templates, suggesting that scale-up of an energy-efficient

synthesis would be easily feasible. These nanoscale EMT materials offer exciting opportunities for both fundamental study and potential industrial applications. The possible green mass production of EMT-type zeolite provides excellent opportunities for applications in catalysis, adsorption, and separations involving larger molecules and for designing thin films, membranes, or nanoscale devices.

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Supporting Online Material

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An Exhumation History of Continents over Billion-Year Time Scales

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The continental lithosphere contains the oldest and most stable structures on Earth, where fragments of ancient material have eluded destruction by tectonic and surface processes operating over billions of years. Although present-day erosion of these remnants is slow, a record of how they have uplifted, eroded, and cooled over Earth's history can provide insight into the physical properties of the continents and the forces operating to exhumate them over geologic time. We constructed a continuous record of ancient lithosphere cooling with the use of uranium-lead (U-Pb) thermochronology on volcanically exhumed lower crustal fragments. Combining these measurements with thermal and Pb-diffusion models constrains the range of possible erosion histories. Measured U-Pb data are consistent with extremely low erosion rates persisting over time scales approaching the age of the continents themselves.

The preservation of fragments of stable Archean continental lithosphere, or “cratons,” over geologic time is intimately linked with the presence of a low-density mantle root that supports and protects the overlying crust (1). The long-term stability of these roots has been attributed to an apparent “isopycnic”

balance between the negative thermal buoyancy from contraction during cooling and the positive chemical buoyancy from the depletion of the root's denser basaltic component during craton formation (1, 2). Despite this stability, cratons must survive exposure to surface processes working to erode on durations lasting billions

of years, a process that results in continued rock exhumation toward Earth's surface. Although present-day erosion within these stable regions is low, the assembly of continental masses through mountain-building processes (3) requires that these terranes experienced periods of rapid erosion after the construction of topographically high mountain belts. An erosional history recording the duration of this early rapid erosional phase and the timing and rate of transition to the slow erosion observed today will allow us to understand more about the composition and density of the lithosphere, its relationship with the underlying mantle, and the thermal, buoyant, and mechanical forces operating to exhumate or bury the continents over the geologic history of Earth.

Because the exhumation or burial of Earth's surface has a direct effect on the rate of heat loss within the lithosphere, a continuous record

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of lithosphere exhumation can be reconstructed through the use of a temperature-sensitive radio-metric dating technique known as thermochronology. The combination of thermochronologic data with thermal models for heat transfer in the lithosphere can be used to measure the processes operating to cool or heat the lithosphere in the geologic past (4). Thermochronologic studies have typically used geochronologic systems sensitive to cooling at temperatures below 110°C. These techniques are most useful for the measurement of cooling attributable to deformation in the upper crust and erosion of topography (5). A thermochronologic system sensitive to cooling at higher temperatures and greater depths is insensitive to the “noise” as-

sociated with near-surface cooling and therefore provides a measure of the background rate of erosion or burial associated with the vertical motions of a craton. The U-Pb thermochronologic system is sensitive to cooling at temperatures of ~400° to 650°C, corresponding to lower crustal depths in cratonic regions of ~20 to 50 km (6). Here, we used this technique to reconstruct an ancient and long-lived thermal history of volcanically exhumed lower crustal fragments—samples that resided at depth for billions of years before recent volcanism transported them to the surface as xenoliths. A high-fidelity reconstruction of time-temperature paths for these samples is produced using the U-Pb system's dual decay scheme, where parent isotopes ^{238}U and ^{235}U

decay at different rates to daughter isotopes ^{206}Pb and ^{207}Pb , respectively. Coupling this dual isotopic system with diffusion's length scale dependency, which causes different crystal sizes to retain Pb over different absolute time scales, results in a set of daughter isotopic compositions for a range of crystal sizes that is unique to the time-temperature history of the sample (7). The measured and modeled U-Pb results presented here explore a range of crystal grain sizes to exploit these advantages (8).

The thermal processes operating to cool or heat the deep lithosphere include conductive heat loss, heat input from the underlying mantle, heat production from the decay of heat-

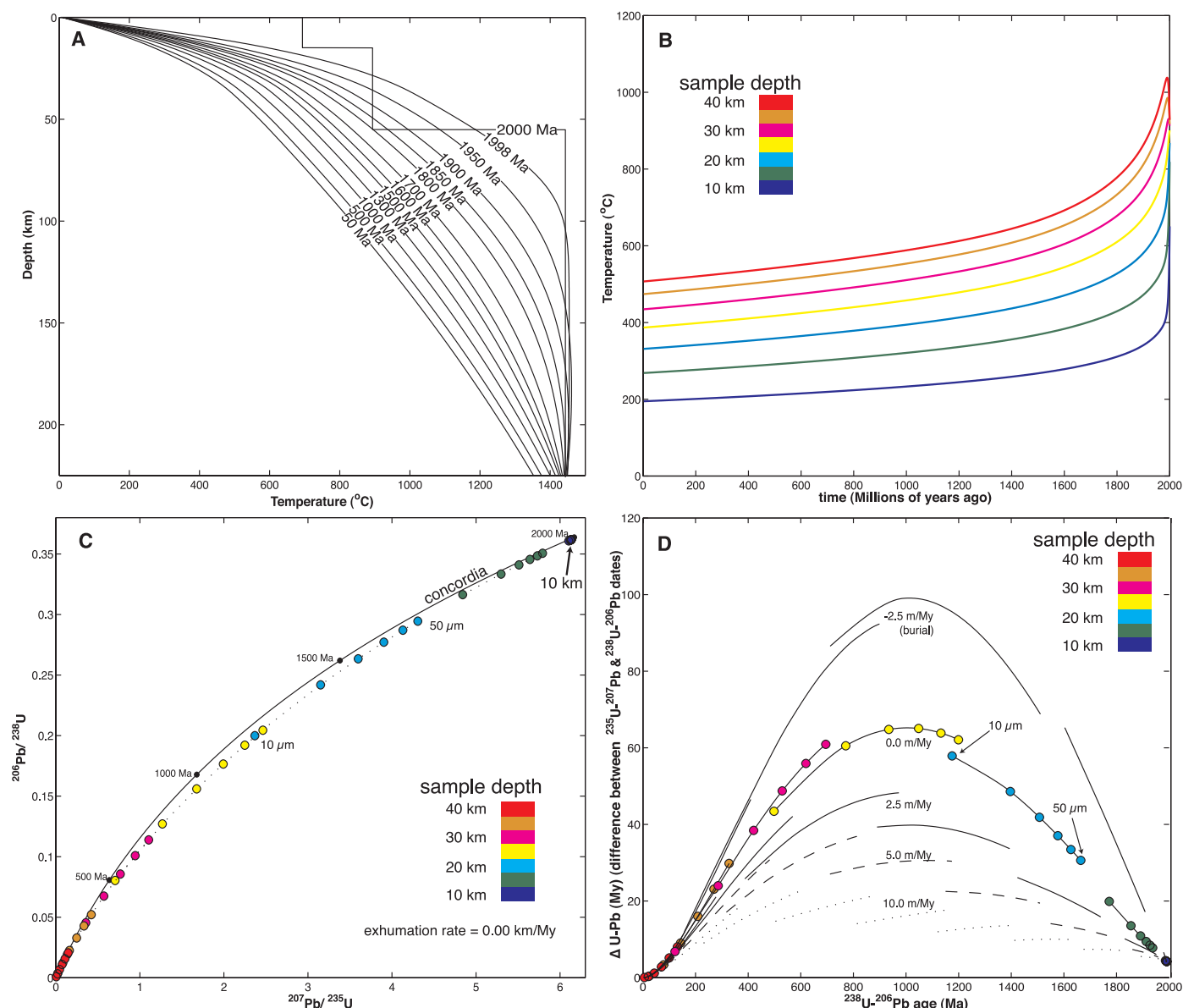


Fig. 1. (A and B) Thermal history for the lithosphere (A) used to produce time-temperature paths (B) for samples at middle to lower crustal depths. (C and D) Time-temperature paths are used to calculate modeled U-Pb thermochronologic data for each sample depth (colors) and over a range of grain sizes. The “concordia” curve in (C) represents the daughter-to-parent ratios of ^{235}U - ^{207}Pb and ^{238}U - ^{206}Pb for a U-Pb system that remains closed throughout Earth history. The shallowest samples cool

quickly through the Pb partial retention zone (PRZ), yielding old ^{238}U - ^{206}Pb dates that plot on concordia (C) or yield $\Delta \text{U-Pb}$ values close to zero (D). The deepest samples reside at temperatures that are too hot for Pb retention in rutile, and thus yield younger dates. The middle to lower crust spends a long time in the Pb-PRZ, resulting in discordance (C) and a large apparent offset between the two U-Pb systems (D). The magnitude of $\Delta \text{U-Pb}$ values (D) is correlated with lithosphere exhumation rate.

producing elements (HPEs), and cooling or insulation due to surface erosion or burial, respectively. The combined effects of these pro-

cesses on the U and Pb isotopic evolution of a set of rutile crystals can be described with a simple thermal model (8). The modeled litho-

sphere thermal history begins with a steep geothermal gradient consistent with formation or reheating during mountain-building events, followed by cooling due to heat loss at the lithosphere surface (Fig. 1A). The time-temperature histories for crustal depths from the thermal model (Fig. 1B) are then used as the input to a model of Pb production and diffusion (8). Modeled U-Pb thermochronologic data correlate directly with sample depth (Fig. 1C). Shallow samples cool quickly through the 400° to 600°C rutile thermal window, yielding U-Pb dates that are consistent with the model start time, whereas the deepest samples never cool below the Pb closure for rutile, accumulate no radiogenic Pb, and yield U-Pb dates of 0 million years ago (Ma) (Fig. 1C). The faster-cooling, shallow samples yield dates that plot on the “concordia” curve (which represents the daughter-to-parent ratios of ^{235}U - ^{207}Pb and ^{238}U - ^{206}Pb for a U-Pb system that remains closed throughout Earth history), indicating agreement between the ^{238}U - ^{206}Pb and ^{235}U - ^{207}Pb systems and closed-system behavior. Samples at intermediate depths accumulate different amounts of radiogenic Pb, depending on depth, forming a curvilinear “discordant” array off the concordia curve that indicates partially open system behavior. Mineral grains that spend a long time in the Pb partial retention zone (PRZ)—a temperature range in which the diffusion and production of radiogenic daughters are at or near a balance—result in the partial retention of Pb with a range of isotopic compositions ($^{207}\text{Pb}/^{206}\text{Pb}$). A whole-crystal analysis yields an integrated measure of the internal Pb-diffusion profile, resulting in the apparent difference between the measured ^{235}U - ^{207}Pb and ^{238}U - ^{206}Pb dates (hereafter referred to as Δ U-Pb). Slower cooling, and thus longer durations in the PRZ, result in a Pb diffusion profile containing a wider range of $^{207}\text{Pb}/^{206}\text{Pb}$ compositions, and thus a greater Δ U-Pb age difference (Fig. 1D) (7). Because the vertical advection of lower crustal rocks toward Earth’s surface increases the rate of cooling at depth, there is a strong correlation between the maximum Δ U-Pb value and net exhumation, where higher net exhumation rates yield lower Δ U-Pb values (Fig. 1D). The U-Pb system’s sensitivity to cooling rate—and, in this thermal setting, to exhumation rate—is the key factor that allows constraints to be placed on the long-term evolution of continental surfaces.

Along the southwestern edge of the North American Craton within Montana, the Archean Medicine Hat Block and Wyoming Province collided at ~1800 Ma to form the Great Falls Tectonic Zone (GFTZ), a Proterozoic suture between the two terranes (9) (Fig. 2B, inset). Lower crustal xenoliths used in this study were collected from four ~50 Ma volcanic epicenters within each terrane. The volcanic entrapment of samples at 50 Ma is so rapid that U/Pb loss due to volcanic reheating is negligible (7). Lower crustal U-Pb thermochronologic data yield the “humped” topology of Δ U-Pb data predicted by

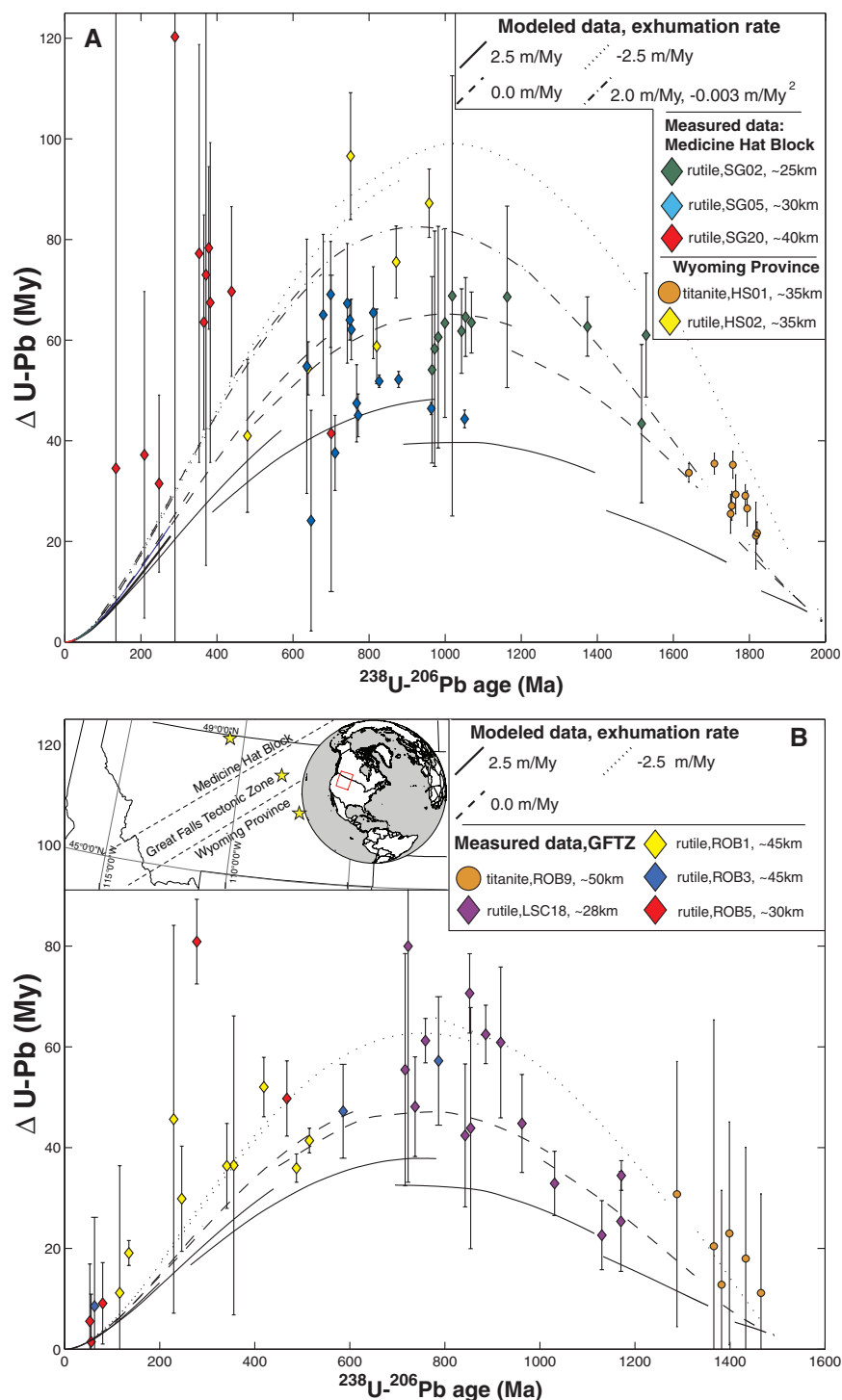


Fig. 2. Plots of Δ U-Pb versus time for measured and modeled lower crustal xenolith data from a northwest-southeast transect through Montana: (A) Archean Medicine Hat Block and Wyoming Province, (B) Proterozoic Great Falls Tectonic Zone (GFTZ). Sample numbers and geobarometrically determined depths are indicated. Faster-cooling, shallow samples yield older dates with a trend of increasing Δ U-Pb in time; deeper samples decrease with time, consistent with the results predicted by the thermal model (Fig. 1D). Uncertainties on individual analyses are shown at the 2σ level and are dominated by the uncertainty of the ^{235}U - ^{207}Pb date. Dashed, dotted, and solid lines mark the forward-modeled U-Pb results with exhumation rates of -2.5 to 2.5 m/Myr. A dashed-dot line in (A) marks the data produced by an erosional history that decreased in time. The inset in (B) shows a simplified geologic terrane map of Montana; stars mark the xenolith sample location within each geologic terrane.

the thermal model (Fig. 1D), supporting the use of this model to infer lithosphere exhumation rate (Fig. 2). The onset of cooling in both regions is recorded by the higher-temperature titanite U-Pb thermochronometer. Within the Archean regions this occurs at ~2000 Ma, indicating the diffusive loss of an Archean cooling record during the younger reheating event (Fig. 2A). Within the Proterozoic GFTZ, the onset of cooling in the region does not occur until ~300 million years (My) after the formation of the inferred mountain belt—a delay in cooling that is consistent with rapid erosion rates between 1800 and 1500 Ma (Fig. 2B). The subsequent long-term cooling history in each terrane is recorded by the lower-temperature rutile thermochronometer, where analyses from xenoliths of different depths provide a continuous and overlapping cooling record lasting more than ~1500 My (Fig. 2). Thermobarometrically determined xenolith depths (7, 10) correlate with the timing of cooling, with the shallowest samples cooling first and deeper samples cooling at progressively younger times (Fig. 2). Assuming an intermediate lithosphere thickness of 225 km (11–13) and the recommended HPE concentrations from compiled data sets (14, 15), forward-modeled rutile data for a range of exhumation rates from –2.5 to 2.5 m/My (where a negative exhumation rate corresponds to burial) bracket the measured data (8) (Fig. 2). Secular variations in exhumation rate control the symmetry of the curves of Δ U-Pb versus time. For example, decreasing the exhumation rate over time skews the peak to the left, yielding modeled data that are also consistent with the measured results, yet their average exhumation rates are bracketed by those that assume constant exhumation (Fig. 2A).

Although the long-term exhumation rates reported here (–2.5 to 2.5 m/My) are in good agreement with shorter-duration observations of some of the slowest-eroding surfaces on Earth (16), they are far slower than what is observed for the majority of continental surfaces (50 to 500 m/My) (16). This disparity can be attributed to the difference in the time scales of observation and secular variations in exhumation. The exhumation rates presented here are long-term integrated estimates and do not preclude the occurrence of brief periods of faster exhumation associated with tectonic (16) or climatic forces (17) operating over intervals much shorter than the observational time scale for this technique. Once a continental mass is laterally isolated from active plate boundaries and vertically supported by a thickened mantle root, the long-term uplift of a mountain belt is more likely dominated by the relative densities of the lithosphere and mantle (isostasy) (18) with a potentially transient and smaller contribution to uplift or burial imposed by density and thermal anomalies in the underlying convecting mantle (dynamic topography) (19).

The U-Pb system's sensitivity to the magnitude and time variability of exhumation rate can be used to set limits on the timing and duration of

events that exhumed or buried the surfaces of continents in the deep geologic past. First, we can gauge the duration of rapid erosional unroofing after mountain building from the difference between collision age and onset of cooling. Within the GFTZ (Fig. 2B), the 300-My difference between the onset of cooling and the mountain-building event establishes a maximum interval over which isostatically driven uplift can persist at the rates observed in young and topographically high mountain belts (50 to 100 m/My) (18). Second, we can rule out erosional histories that include periods of exhumation or burial faster than a given rate or longer than a given time interval (with a trade-off between duration and rate). For example, we can dismiss histories for the GFTZ with exhumation or burial lasting longer than 50 My at rates that exceed ± 50 m/My. More subtle transient deformation mechanisms such as dynamic topography, with a characteristic amplitude less than 1 km over a distance of 1000 km or more, will be undetected on the short time scales (tens to hundreds of millions of years) and modest exhumation/burial rates (10 to 100 m/My) at which this process operates (19). Despite the possibility of brief erosional or burial events of moderate magnitude, the data presented here require an overall cratonic history dominated by vertical motion rates near zero. This indicates that the isostatic balance observed in the present-day continents has been largely maintained over geologic time, extending back at least to the onset of cooling within each terrane. Since this stability was first attained, the craton has experienced a balance between erosion and burial, with a corollary balance between the lithosphere's internal buoyancy forces (1, 2) and near-zero isostatic uplift, further indicating a minimal change in the relative densities of the lithosphere and mantle over intervals lasting billions of years.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/335/6064/73/DC1
Materials and Methods
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Tables S1 and S2
References (20–28)

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Multiyear Prediction of Monthly Mean Atlantic Meridional Overturning Circulation at 26.5°N

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Attempts to predict changes in Atlantic Meridional Overturning Circulation (AMOC) have yielded little success to date. Here, we demonstrate predictability for monthly mean AMOC strength at 26.5°N for up to 4 years in advance. This AMOC predictive skill arises predominantly from the basin-wide upper-mid-ocean geostrophic transport, which in turn can be predicted because we have skill in predicting the upper-ocean zonal density difference. Ensemble forecasts initialized between January 2008 and January 2011 indicate a stable AMOC at 26.5°N until at least 2014, despite a brief wind-induced weakening in 2010. Because AMOC influences many aspects of climate, our results establish AMOC as an important potential carrier of climate predictability.

Variations in Atlantic Meridional Overturning Circulation (AMOC) can substantially affect northward ocean heat

transport and therefore European and North Atlantic climate (1–3). Through its influence on sea surface temperature (SST), AMOC is further-

more thought to influence climate phenomena such as Sahel droughts and North Atlantic hurricane activity (4–6). In the near term (interannual to decadal time scales), AMOC and other climate variations are influenced by the combination of anthropogenic forcing, natural forcing, and internal variability; near-term climate predictions must hence be started (initialized) from the present ocean state reflecting the present phase of internal variability. Multiyear climate predictions have, however, so far been limited to predicting surface temperature variations (7–10) and hurricane frequency (11); whether there is multiyear prediction skill for any element of the large-scale atmospheric or oceanic circulation has remained unclear. This paper demonstrates prediction skill for AMOC at 26.5°N for up to 4 years in advance.

Owing to a dearth of long-term AMOC observations, AMOC predictability has hitherto been addressed exclusively in a “perfect model” framework. Results of a model simulation were used as a surrogate for observations, and the degree of similarity between the surrogate observations and an initialized simulation was interpreted as an estimate of AMOC predictability. These studies have shown that AMOC strength is potentially predictable for up to a decade, with potential skill varying among different models (12–15). Here, we take advantage of the first half-decade-long observed estimate of AMOC at 26.5°N [the RAPID-MOCHA (Meridional Overturning Circulation and Heatflux Array) projects (16, 17)] to quantify the predictive skill of initialized multiyear predictions performed with the ECHAM5/MPI-OM coupled climate model (18). In further contrast to previous work, we focus on the monthly varying AMOC instead of multiyear averages; this focus is warranted both because of potential applications such as hurricane predictions (11) and because there is clear model evidence that European climate is influenced by year-to-year changes in AMOC (3).

At multiyear to decadal time scales, the memory and, hence, the potential for predictability of the climate system are thought to reside in the ocean. The historical subsurface ocean data are limited in both space and time, and some have been recently shown to be systematically biased (19). Moreover, the current ocean reanalyses display a broad spectrum of AMOC mean state and variability (20, 21), although they use the same observational database. Therefore, we take an alternative route to ocean reanalysis and initialize the coupled model European Centre Hamburg Model 5 (ECHAM5)–Max Planck Institute Ocean Model (MPI-OM) from an ensemble of MPI-OM ocean-only runs that are forced with the atmospheric state of the National Centers for

Environmental Prediction–National Center for Atmospheric Research reanalysis (22) for the period 1948 to 2010 (23). We thus ensure dynamical consistency of the forecast model and the initialized ocean state; moreover, forced experiments with MPI-OM have already been used successfully to reconstruct the observed variability of the Nordic Seas Overflows (24), a main contributor to North Atlantic Deep Water that feeds the lower limb of AMOC.

The skill of any prediction system is assessed by using it retrospectively; forecasts are made employing observations only up to some point in the past, and the time period after that point is then used to evaluate the quality of this retrospective forecast (“hindcast”). We have performed ensemble hindcasts starting in January of each year from 2004 to 2007 (23). AMOC strength in the hindcasts closely follows the observations for up to 4 years; the best resemblance to the phase of the observed variability is simulated by the hindcast ensemble mean (Fig. 1). Visual inspection already shows that the hindcast quality is not the same for every start year, a notion now made quantitative.

We measure the skill of the hindcasts primarily with the anomaly correlation coefficient (COR) between the observations and the individual hindcasts or their ensemble mean. We then compare COR against the benchmark skill of an ensemble of noninitialized model simulation (hereafter ens20C) and of the persistence, a commonly used statistical forecast (23). We consider lead times of 1 to 4 years within each of the 10-year hindcasts and find that, for many start dates, AMOC variations are predicted with significantly greater skill by the initialized hindcasts than by the persistence forecast for all lead times (Fig. 2). The ensemble-mean hindcasts initialized in each January of 2004 to 2006 have mostly high COR, ranging between 0.8 and 0.95 for year 1, 0.5 to 0.8 for year 2, around 0.3 to 0.8 for year 3, and 0.45 to 0.55 for year 4. In contrast, the start date January 2007 gives only low COR skill around 0.3. The ens20C ensemble mean obtains low COR skill of 0.4.

We diagnose skill improvement through initialization not only from higher COR but also from a tendency toward a more realistic amplitude of AMOC fluctuations (Fig. 1). We note that the hindcast ensemble mean underestimates the observed variability to a larger degree than do the individual hindcasts; conversely, the hindcast ensemble mean has considerably higher COR skill than individual hindcasts (fig. S1). Persistence forecasts, by contrast, do very well on AMOC amplitude but usually lose COR skill after 1 year (Fig. 2 and fig. S2).

The hindcast time series (Fig. 1) clearly show that the dominant AMOC signal that is captured better by the hindcasts than by ens20C arises from the seasonal cycle. The observed time series is too short to distinguish robustly between the mean seasonal cycle and monthly mean deviations thereof; the standard error of the seasonal

AMOC is ± 2 Sv (25), which is close to the typical monthly mean AMOC deviation from the mean seasonal cycle. Therefore, we cannot at present distinguish between predictability of climatological and anomalous seasonality.

Because it is AMOC seasonality that is most clearly improved through our initialization, one might argue that this particular skill increase is not difficult to achieve. We counter this challenge by noting that, unlike the seasonal cycle in temperature, the observed AMOC seasonal cycle, with a maximum in October and a minimum in April, has highly nontrivial causes (25, 26). The largest contribution to observed AMOC seasonality arises from the baroclinic upper-mid-ocean transport between the Bahamas and Africa, which in turn is dominated by density variations near the eastern boundary (25, 26). These seasonal density variations are coherent to depths of 1400 m (26), implying a substantial role of internal ocean dynamics in the seasonal cycle of AMOC. These observational facts both give credence to our claim of enhanced AMOC hindcast skill and suggest that AMOC predictability chiefly arises from the predictability of the upper-mid-ocean transport.

To investigate this suggestion, we first note that AMOC at 26.5°N can be estimated as the

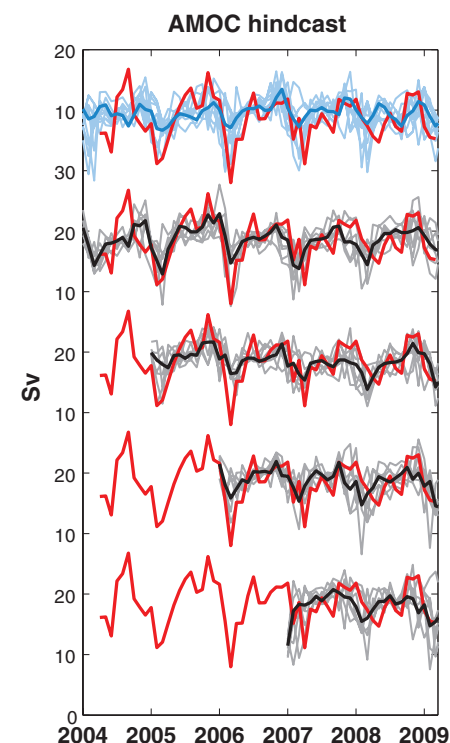


Fig. 1. Multiyear AMOC hindcasts compared with observations and noninitialized climate model simulations. AMOC strength is the zonally integrated northward flow at 26.5°N above 1000-m depth. The observed transports are from RAPID/MOCHA (red); the individual hindcasts and their ensemble mean are shown in gray and black, respectively. AMOC from the noninitialized climate simulations (ens20C) is shown in the upper plots in light blue for individual experiments and dark blue for the ensemble mean.

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sum of three independently obtained transport components: upper-mid-ocean transport (MO), Ekman transport (EK), and Florida Current transport (FC) (16, 17, 27). MO is derived from thermal wind balance (23). We find that skilful hindcasts of MO can be made for up to 3 years (Fig. 3A), and we find much less sensitivity to start year; MO prediction is more robust than is AMOC prediction. MO skill improvement is also documented in the generally higher and more realistic

amplitude of hindcast variability, compared to ens20C (figs. S3 and S4).

In both observations and hindcasts, we see a very strong connection between MO and the zonal density difference across the basin, averaged over the depth range 200 m to 1000 m ($\Delta\rho_u$) (fig. S5). We find in our hindcasts significant COR skill for $\Delta\rho_u$ for up to 3 years (Fig. 3B and fig. S5). This skill both explains the origin of the MO hindcast skill and makes plausible the

greater robustness of MO hindcast skill for different start years when compared with AMOC hindcast skill. AMOC hindcast skill is also influenced by EK and FC, both of which are less predictable than MO (fig. S6 and SOM text). Hence, EK and FC can diminish the AMOC hindcast skill, as is the case for the hindcasts started in January 2007, or enhance it, as is the case for the hindcasts started in January 2004 and 2005 (fig. S6 and SOM text).

The observed seasonal cycles in AMOC and eastern-boundary density at 26.5°N are thought to be driven primarily by seasonal wind-stress-curl variations near the eastern boundary (25, 26). To find multiyear predictability for a wind-driven climate quantity might appear surprising, because we do not expect predictability for wind stress itself. We speculate, however, that the seasonally varying wind-stress curl near the eastern boundary might repeatedly imprint itself onto eastern-boundary density. Hence, knowledge of density at any given time would reflect forcing over a longer period in the past, and predictive skill for density would not imply predictive skill for wind stress.

With hindcast skill established, we are now in a position to produce AMOC forecasts. For each January from 2008 until 2011, we construct an ensemble of nine forecasts spanning 10 years. We consider all these simulations forecasts because the RAPID/MOCHA estimate is currently only available until the beginning of April 2009, and the period of overlap is very short. The existence of such a conceptual “gray zone”—where the ocean model forcing and, hence, our initialization as well as EK are known but MO is

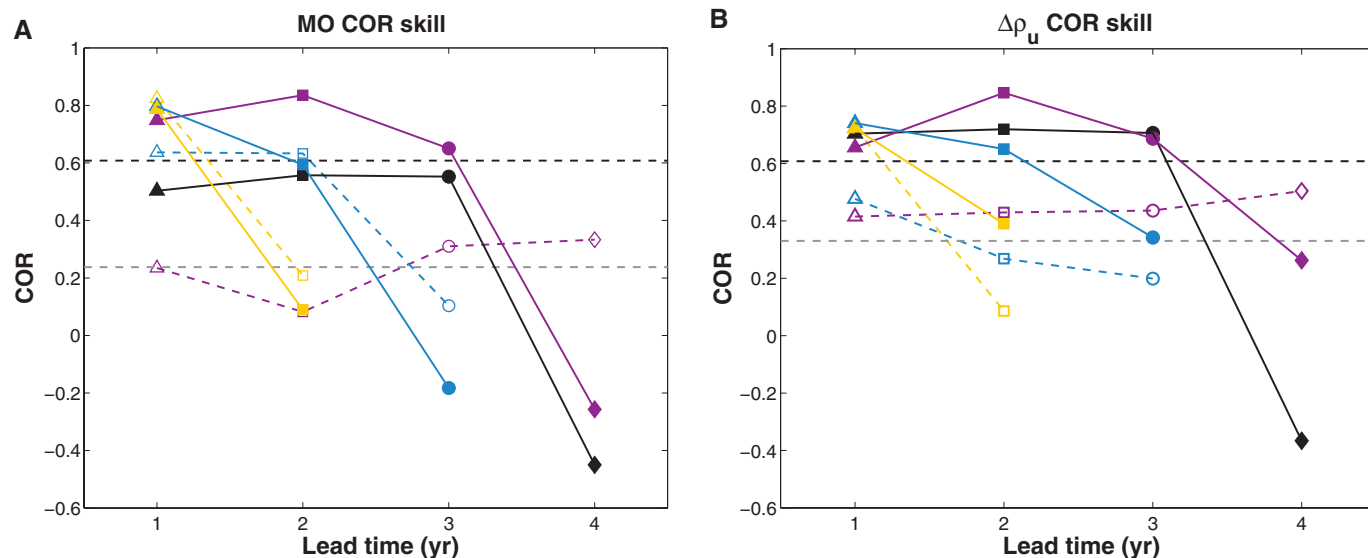
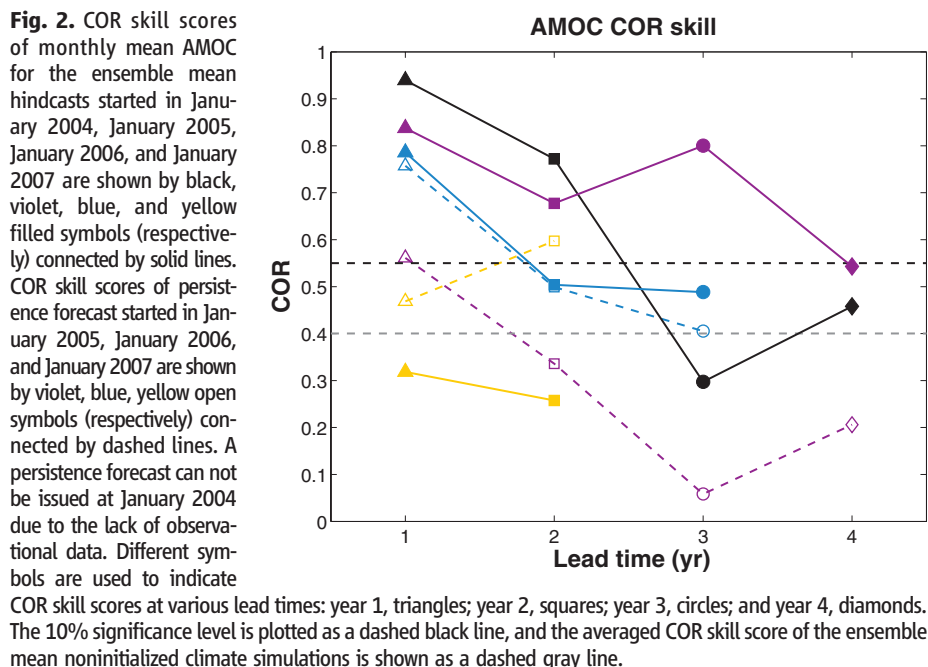


Fig. 3. COR skill scores of monthly mean MO (A) and upper ocean zonal density difference ($\Delta\rho_u$) (B). COR skill scores for the ensemble mean hindcasts started in January 2004, January 2005, January 2006, and January 2007 are shown by black, violet, blue, and yellow filled symbols (respectively) connected by solid lines. COR skill scores of persistence forecast started in January 2005, January 2006, and January 2007 are shown by violet, blue, and yellow open symbols (respectively) connected by dashed

lines. A persistence forecast can not be issued at January 2004 due to the lack of observational data. Different symbols are used to indicate COR skill scores at various lead times: year 1, triangles; year 2, squares; year 3, circles; and year 4, diamonds. The 10% significance level is plotted as a dashed black line, and the averaged COR skill score of the ensemble mean noninitialized climate simulations is shown as a dashed gray line.

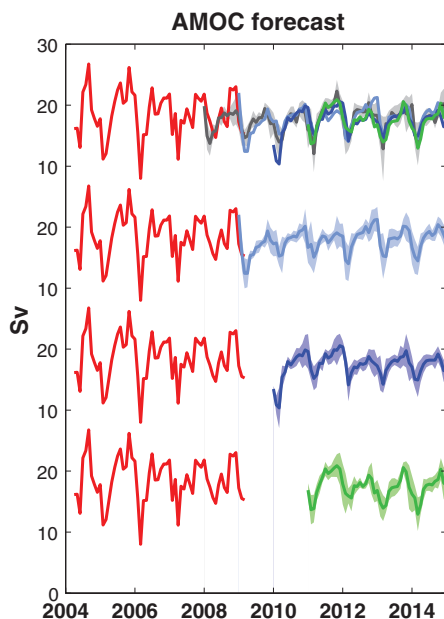


Fig. 4. Multiyear predictions of AMOC transport. RAPID/MOCHA time series are shown in red; ensemble mean forecasts are shown in dark gray, light blue, dark blue, and green for the forecasts starting in January 2008, January 2009, January 2010, and January 2011, respectively. The pale shading represents the 95% confidence intervals of the nine-member forecast ensemble initialized in January 2008, January 2009, January 2010, and January 2011.

not—is inevitable for as long as in situ ocean interior measurements cannot be made in real time.

For all start years, the ensemble-mean forecasts until 2014 indicate a generally stable AMOC (Fig. 4). However, the forecast initialized in March 2010 shows a pronounced AMOC minimum in March 2010 that arises from a minimum in EK (fig. S7),

which in turn is induced by an extremely negative North Atlantic Oscillation in winter 2009–2010 (28). The real AMOC minimum in March 2010 may turn out to be even deeper than predicted, because our ensemble mean underpredicts AMOC amplitude (fig. S2). We are confident, however, that the AMOC minimum in March 2010 will be a short-lived phenomenon; our confidence is based on the insensitivity of our AMOC and MO forecasts to the start year.

We cannot readily generalize our results for 26.5°N to other latitudes; recent studies reported a change in the character of AMOC fluctuations around 40°N, with a strong decadal component to the north and enhanced higher-frequency variability to the south (29–31). However, for 26.5°N, we have established AMOC hindcast skill, we understand that this skill arises from the mid-ocean transport, and we confidently predict a stable AMOC at least until the end of 2014. Moreover, our findings demonstrate that skill in climate prediction arises not only from the large ocean thermal inertia but potentially also from the long time scales of internal ocean dynamics.

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Supporting Online Material

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Ancestral Developmental Potential Facilitates Parallel Evolution in Ants

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Complex worker caste systems have contributed to the evolutionary success of advanced ant societies; however, little is known about the developmental processes underlying their origin and evolution. We combined hormonal manipulation, gene expression, and phylogenetic analyses with field observations to understand how novel worker subcastes evolve. We uncovered an ancestral developmental potential to produce a “supersoldier” subcaste that has been actualized at least two times independently in the hyperdiverse ant genus *Pheidole*. This potential has been retained and can be environmentally induced throughout the genus. Therefore, the retention and induction of this potential have facilitated the parallel evolution of supersoldiers through a process known as genetic accommodation. The recurrent induction of ancestral developmental potential may facilitate the adaptive and parallel evolution of phenotypes.

The wingless worker caste, a universal feature of ants (1, 2), has repeatedly expanded into a complex system of morphologi-

cal and behavioral subcastes. The existence of these subcastes has long fascinated biologists (3–9), yet little is known about their develop-

mental and evolutionary origin (7, 8). The ant genus *Pheidole* is one of the most species-rich genera, with 1100 species worldwide (10, 11). All *Pheidole* species have two worker subcastes: minor workers (Fig. 1C) that perform most tasks in the nest and forage and soldiers (Fig. 1B) that

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defend the nest and process food (11). This complex worker caste system is thought to have promoted the remarkable diversification of *Pheidole* by enhancing the division of labor (11).

In a wild *P. morrisi* colony, we discovered several anomalous soldier-like individuals. These individuals are anomalous because they are significantly larger than normal soldiers (Fig. 2, A and B, and fig. S1), and unlike normal soldiers, they have mesothoracic wing vestiges (Fig. 2, C and D) and rarely occur in nature. These anomalous soldiers are similar to a supersoldier subcaste, which is known to be continually produced in eight *Pheidole* species (fig. S2) (10–12). These species co-occur with army ants and live exclusively in the deserts of the American southwest and northern Mexico (11). In one of these species, *P. obtusospinosa* (Fig. 2, E and I), a major function of the supersoldier subcaste is to block the nest entrance with their extra-large heads and engage in combat to defend against army ant raids (13).

The similarity between the supersoldier-like anomalies in *P. morrisi* and the supersoldier subcaste suggests that they share a developmental origin. Normal soldier development in *Pheidole* may provide insight into how supersoldier-like anomalies may have originated. The soldier subcaste is determined late in larval development at a soldier–minor worker switch point (Fig. 1), which is largely controlled by nutrition (5) and mediated by juvenile hormone (JH) (14, 15). Soldier development is defined by two features: (i) Soldier-determined larvae grow larger than minor worker larvae; and (ii) they develop a pair of vestigial forewing discs in their mesothoracic segment (Fig. 1, E and F) (14–16). These discs show a soldier-specific expression of *spalt* (*sal*) (Fig. 1E) (1), a key gene in the network underlying wing polyphenism in *Pheidole*. *Sal* is a key gene because its expression is spatiotemporally associated with the induction of apoptosis in these vestigial forewing discs (17, 18). Therefore, the supersoldier-like anomalies we found in *P. morrisi* were likely to have originated from the abnormal growth of soldier larvae and their vestigial wing discs. Based on this insight, we predicted that the evolution of the supersoldier subcaste in *Pheidole* occurred through developmental changes that elaborated these two features.

We tested this prediction in *P. obtusospinosa* and *P. rhea*, two species that have a supersoldier subcaste (11). As predicted, their supersoldier larvae grow larger (Fig. 2, F and J, and fig. S3, B and F) and develop two pairs of large vestigial wing discs relative to their soldier larvae (Fig. 2, G and K, and fig. S3, C and G). Furthermore, vestigial wing discs in supersoldier larvae show an elaborated pattern of *sal* expression in the wing pouch relative to those of soldier larvae (Fig. 2, H and L, and fig. S3, D and H, black arrows). We then resolved the evolutionary history of their supersoldier subcaste by reconstructing a phylogeny of 11 *Pheidole* species (fig. S5). Of these, only *P. obtusospinosa* and *P. rhea* have a supersol-

dier subcaste. Our phylogenetic analysis suggests that the supersoldier subcaste has evolved independently, because *P. rhea* is one of the most basal species of this genus, whereas *P. obtusospinosa* is derived (fig. S5) (10). Therefore, the supersoldier subcaste has evolved in parallel, because similar developmental changes underlie its inde-

pendent evolution in *P. obtusospinosa* and *P. rhea*. Furthermore, our phylogenetic analysis suggests that, relative to *P. obtusospinosa*, there are six basal and four derived species (fig. S5). We found that soldier larvae of these basal and derived species differ in their vestigial wing disc number and wing pouch expression of *sal* (fig. S6). This indi-

Fig. 1. Wing polyphenism in *P. morrisi*: the ability of a single genome to produce (A) winged queens and wingless (B) soldiers and (C) minor workers (2). Caste determination occurs at two JH-mediated switch points in response to environmental cues (1, 15, 30). (D) Wing discs in queen larvae showing conserved hinge and pouch expression of *sal*. (E) Vestigial wing discs in soldier larvae showing a soldier-specific pattern of *sal* expression, where it is conserved in the hinge but down-regulated in the pouch. Asterisks represent the absence of visible wing discs and *sal* expression in (E) soldier and (F) minor worker larvae. Scale bars indicate the relative sizes of queen, soldier, and minor worker larvae and adults.

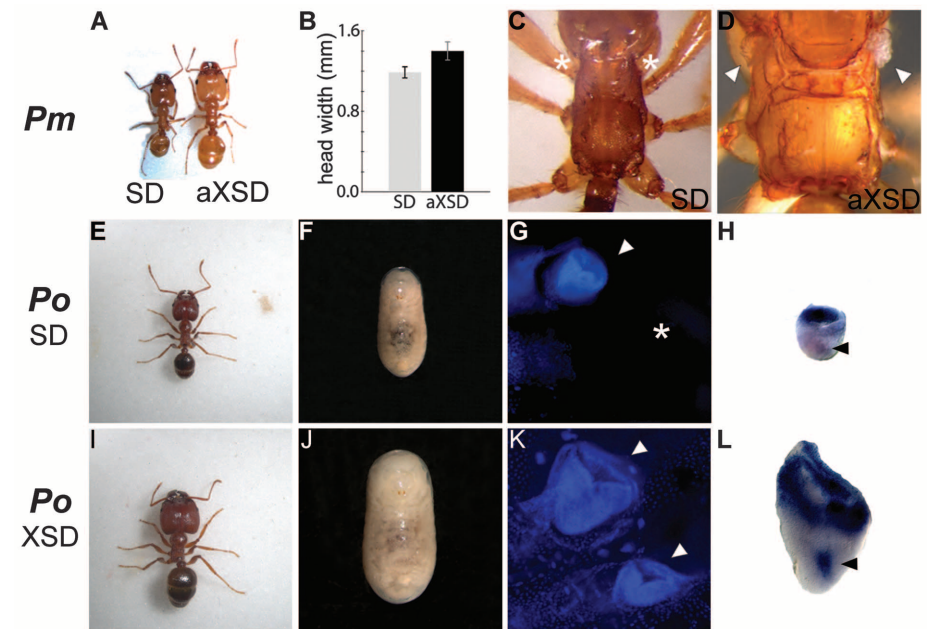
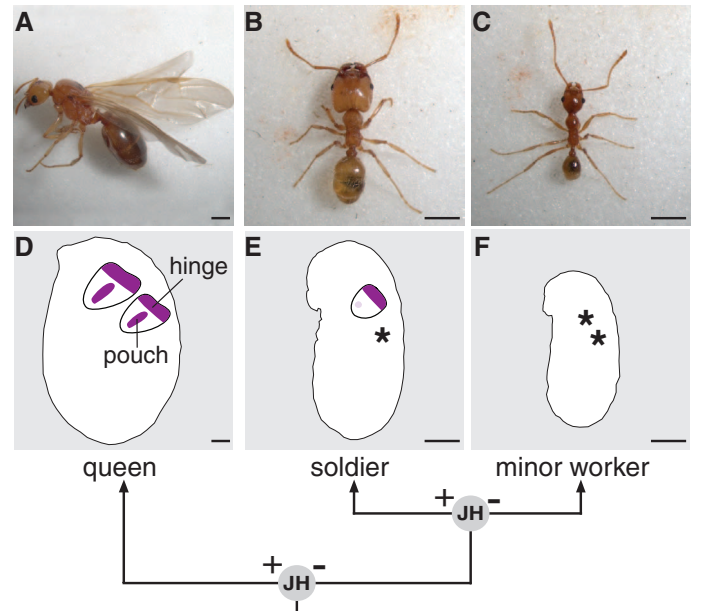


Fig. 2. Comparison of *P. morrisi* (Pm) ants: (A, left) Normal adult soldier (SD) and (A, right) anomalous supersoldier (aXSD). (B) Mean and standard deviation of head width of normal SD (gray) and aXSD (black) (fig. S1), and thorax of (C) a normal SD and (D) an aXSD. Comparison of *P. obtusospinosa* (Po) ants: adults of (E) SD and (I) supersoldier (XSD); larvae of (F) SD and (J) XSD; and vestigial wing discs [stained with 4',6'-diamidino-2-phenylindole (DAPI)] and *sal* expression in SD (G and H and fig. S4A) and XSD (K and L and fig. S4B). White arrowheads indicate the presence of mesothoracic vestigial wing buds or discs; asterisks denote their absence. Black arrowheads indicate *sal* expression in the wing pouch. Adult, larval, and vestigial wing disc images are all to scale. See fig. S3 for a comparison of *P. rhea* SD and XSD.

cates that the supersoldier subcaste has evolved in parallel despite the evolutionary divergence of soldier development.

Application of methoprene (a JH analog) to *Pheidole* larvae has been shown to induce the development of unusually large soldier pupae (15). In *P. morrisi*, we found that methoprene can induce the development of larvae and adults that mimic the anomalous supersoldier-like individuals of *P. morrisi* and the supersoldiers of *P. obtusospinosa* and *P. rhea*. First, induced

supersoldier larvae (Fig. 3G) and adults (Fig. 3B) are significantly larger than untreated controls (Fig. 3, D and A, and fig. S7), and several of the induced adult supersoldiers have mesothoracic wing vestiges (Fig. 3C). Second, the relative size ranges of induced supersoldiers overlap with those of anomalous and naturally produced supersoldiers (fig. S8). Finally, we found vestigial wing discs of induced supersoldier larvae (Fig. 3, H and I, and fig. S9, B to D and F to H) that mimic those of supersoldier larvae in *P. obtusospinosa* (Fig. 2,

K and L) and *P. rhea* (fig. S3, G and H). Therefore, although *P. morrisi* lacks a supersoldier subcaste, there is a developmental potential to produce supersoldiers that can be induced through JH. Furthermore, the occurrence of supersoldier-like anomalies in *P. morrisi* (Fig. 2A) and other *Pheidole* species (16) suggests that this potential is recurrently induced in nature. This recurrent induction, which is probably mediated by JH, may be caused by nutrition, because it has been shown that environmental variation in nutrition (3) and experimentally increasing nutrition (5) produces supersoldier-like anomalies in *Pheidole* colonies.

We discovered that this developmental potential to produce supersoldiers can be induced by methoprene in other derived (*P. hyatti*) and basal (*P. spadonia*) *Pheidole* species that lack a supersoldier subcaste. As in *P. morrisi*, we found vestigial wing discs of induced supersoldier larvae in *P. hyatti* (fig. S9, J and N) and *P. spadonia* (fig. S9, R and V) that mimic those of supersoldier larvae in *P. obtusospinosa* (Fig. 2, K and L) and *P. rhea* (fig. S3, G and H). Therefore, the developmental potential to produce supersoldiers has been retained and was probably present in the common ancestor of all *Pheidole* (Fig. 4). Without a priori knowledge of this ancestral developmental potential, we would have inferred that the supersoldier subcaste has evolved de novo: once in *P. rhea* and once in *P. obtusospinosa* (fig. S5) (10). However, our results support an alternative explanation for the parallel evolution of supersoldiers: The developmental potential and phenotypic expression of a novel supersoldier subcaste originated in the common ancestor of all *Pheidole* (Fig. 4, section i); the phenotypic expression of supersoldiers was subsequently lost, but the ancestral potential to produce them was retained (Fig. 4, section ii); and this potential was then actualized in *P. obtusospinosa*, leading to the re-evolution of a supersoldier subcaste (Fig. 4, section iii).

Finally, we showed that this ancestral potential was actualized in *P. obtusospinosa* through the re-evolution of a second JH-sensitive period mediating a soldier-supersoldier switch point (fig. S10). We applied methoprene to larvae that had passed the soldier–minor worker switch point but whose caste fate as either soldiers or supersoldiers was still undetermined. We found that applying methoprene to these larvae induced the development of a significantly greater proportion of supersoldiers (fig. S11). Collectively, our results indicate that the supersoldier subcaste in *P. obtusospinosa* re-evolved through genetic accommodation. This process occurs when: (i) a novel phenotype is induced and (ii) this phenotype is incorporated into the population through selection on genes that control its frequency and form of expression (19, 20). Environmental induction of the ancestral potential may have recurrently produced supersoldier-like anomalies in *P. obtusospinosa*. These anomalies would persist, because colonies of *Pheidole* generally care for and buffer anomalies against purifying

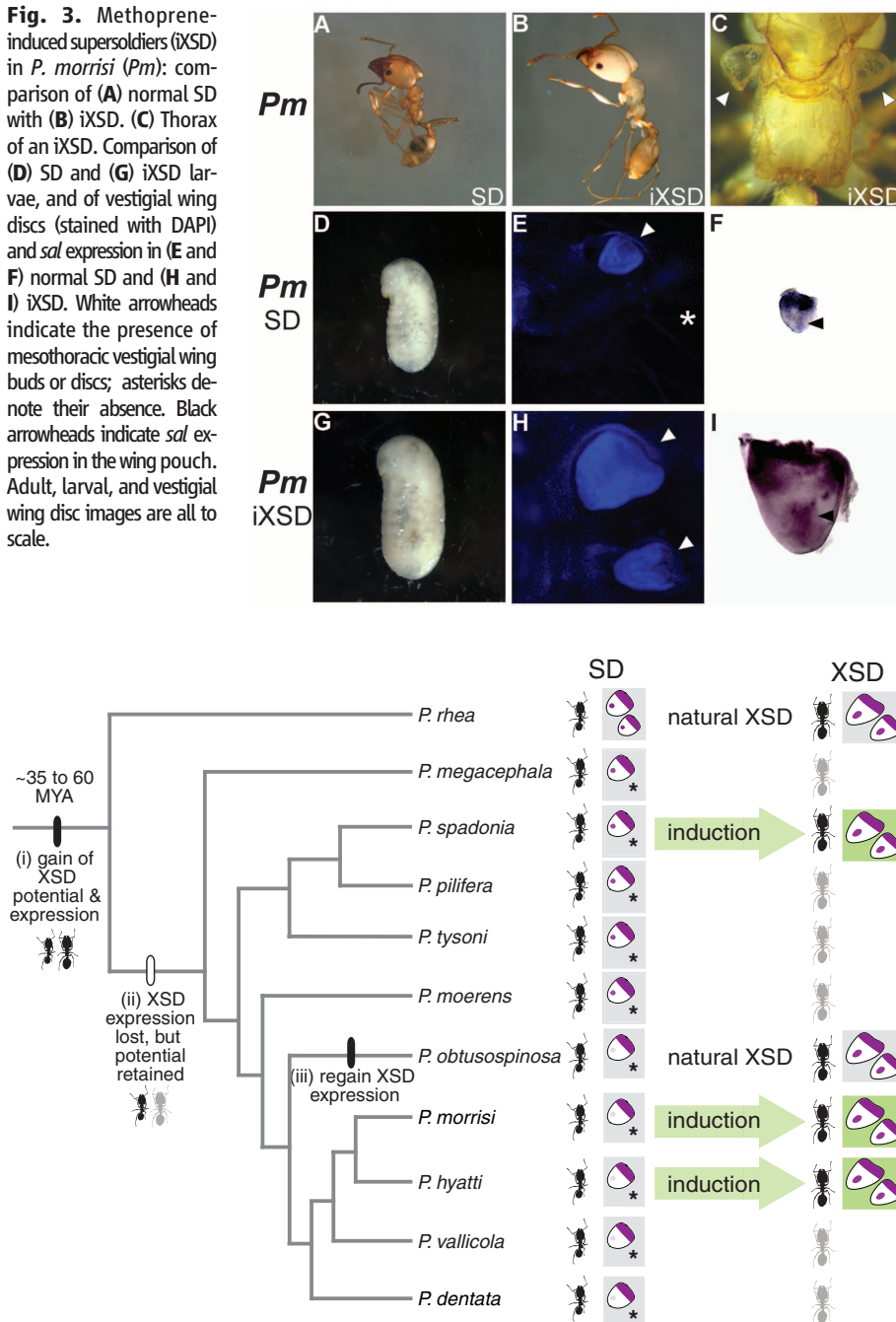


Fig. 4. Evolutionary history of ancestral developmental potential and phenotypic expression of supersoldiers (XSDs). MYA, million years ago. Purple represents the pattern of *sal* expression; asterisks indicate the absence of vestigial wing discs and *sal* expression. Green arrows and boxes represent the induction of XSD potential.

selection (3). Selection on *P. obtusospinosa* colonies may have incorporated induced supersoldier-like anomalies by increasing their frequency through modification of the JH system (fig. S12) and by inhibiting the formation of any wing vestiges (fig. S13 and S14). Army ant raids may have been a selective pressure that incorporated these anomalies, because *P. obtusospinosa* supersoldiers currently use their extra-large heads to defend against these raids (13).

Selection for re-evolving supersoldiers may generally be reduced, because almost all *Pheidole* species lack a supersoldier subcaste (10, 11). *P. hyatti* provides insight into how this selective pressure can be reduced: Although *P. hyatti* retains the developmental potential (Fig. 4) and lives in an ecological environment similar to that of *P. obtusospinosa*, it has not re-evolved a supersoldier subcaste (11). Instead, *P. hyatti* uses nest evacuation behavior when attacked by army ants (21). The retention of this potential in *P. hyatti* and other *Pheidole* species that lack a supersoldier subcaste may therefore be due to a clade-specific constraint (22). This constraint may have arisen from having the same hormone (JH) mediate the determination of both soldiers and supersoldiers in the common ancestor of all *Pheidole*. Soldiers and supersoldiers are both defined by their larval size and the development of their vestigial wing discs, which indicates that their developmental programs share many modules. Therefore, the ancestral potential to produce supersoldiers cannot be lost without compromising the developmental program of soldiers.

Recurrent phenotypes reflecting ancestral potentials have long been recognized as widespread in plants and animals (6, 19, 23–28). Because of the lack of empirical evidence, however, the evolutionary significance of these recurrent phenotypes has been underappreciated (19, 29). We uncovered an ancestral developmental potential to produce a novel supersoldier subcaste that has been retained throughout a hyperdiverse ant genus that evolved ~35 to 60 million years ago (10) (Fig. 4). Our results suggest that the recurrent induction of ancestral developmental potential is an important source of adaptive variation for selection that facilitates the adaptive and parallel evolution of novel phenotypes.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/335/6064/79/DC1
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Figs. S1 to S16
Tables S1 to S2
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Fitness Trade-Offs and Environmentally Induced Mutation Buffering in Isogenic *C. elegans*

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Mutations often have consequences that vary across individuals. Here, we show that the stimulation of a stress response can reduce mutation penetrance in *Caenorhabditis elegans*. Moreover, this induced mutation buffering varies across isogenic individuals because of interindividual differences in stress signaling. This variation has important consequences in wild-type animals, producing some individuals with higher stress resistance but lower reproductive fitness and other individuals with lower stress resistance and higher reproductive fitness. This may be beneficial in an unpredictable environment, acting as a “bet-hedging” strategy to diversify risk. These results illustrate how transient environmental stimuli can induce protection against mutations, how environmental responses can underlie variable mutation buffering, and how a fitness trade-off may make variation in stress signaling advantageous.

A specific mutation can have different consequences in different individuals. For example, even in “Mendelian” human diseases, such as cystic fibrosis, an inherited mutation can result in severe disease in one individual but a milder phenotype in another (1). Incomplete penetrance is also observed in isogenic model organisms and is poorly understood (2–4).

Many mutations have outcomes that depend on the activity of molecular chaperones—

proteins that aid the folding of other macromolecules (5–14). More generally, molecular mechanisms that promote environmental robustness (survival after environmental challenges) also tend to increase mutational robustness [the extent to which an organism’s phenotype remains constant in spite of mutation (15–17)].

We investigated whether genetically increasing environmental stress resistance could modify mutation penetrance in the model organism

Caenorhabditis elegans. We used a transgene to overexpress the transcription factor heat shock factor 1 (HSF-1), a master regulator of the environmental stress response. Transgenic animals are more resistant to a range of environmental challenges (18, 19) and show a delayed age-dependent reduction in protein-folding homeostasis (20). We crossed the *hsf-1* transgenic animals with strains carrying diverse mutations that affect development but with outcomes that vary across individuals (table S1).

In 8 out of 11 tested cases, mutation penetrance was reduced in the transgenic animals (Fig. 1, fig. S1, and table S2). Protection was observed for mutations affecting both embryonic (Fig. 1A) and postembryonic (Fig. 1B) development. For example, embryonic lethality caused by a deletion in the intermediate filament protein gene *ifb-1* reduced from 33% to 17% (48% of animals that would have died were protected, $P = 5.7 \times 10^{-12}$) (Fig. 1, fig. S1, and table S4). The buffered mutations are molecularly diverse and act in distinct

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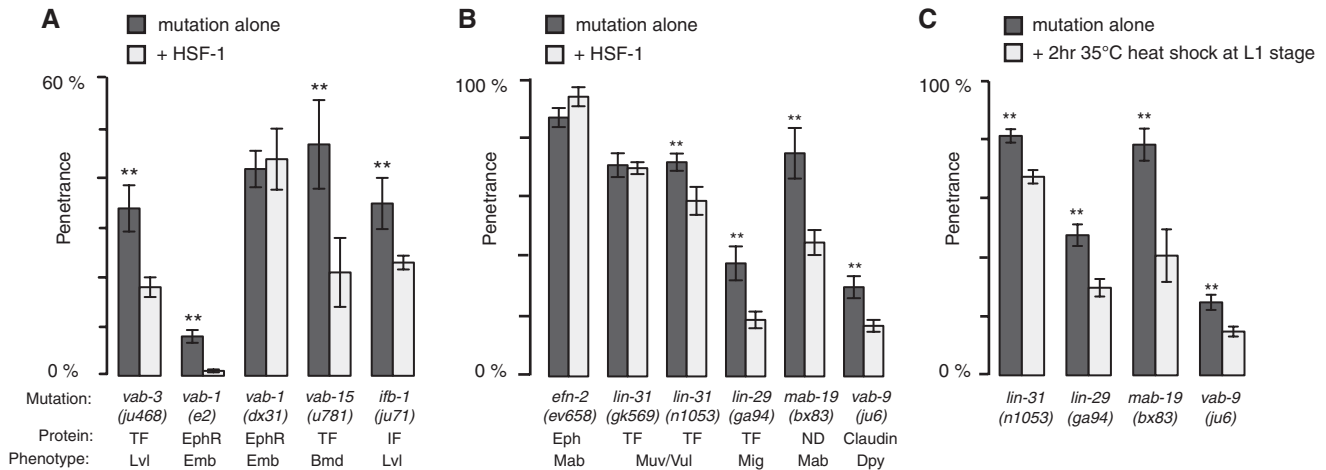


Fig. 1. Genetic and environmental stimulation of mutation buffering during the development of *C. elegans*. Increased expression of HSF-1 reduces the penetrance of mutations acting early (A) or late (B) in development. Similarly, a 2-hour 35°C heat shock at the L1 stage of development reduces the penetrance of late-acting mutations (C). ** $P < 0.01$, Fisher exact test; error

bars indicate SEM. Phenotypes: Lvl, larval lethal; Emb, embryonic lethal; Bmd, body morphology defect; Muv, multivulva; Vul, vulvaless; Mig, male gonad migration defect; Mab, male abnormal (male tail ray defect); Dpy, dumpy; TF, transcription factor; EphR, ephrin receptor; IF, intermediate filament; ND, not determined. See also fig. S1, tables S1 to S5.

pathways and tissues (table S1). Protection ranged from 18 to 88% in the different cases. All of the buffered mutations had temperature-sensitive outcomes, whereas those refractive to buffering did not, and they likely represent genetic nulls (tables S1 and S5).

These observations suggest that, at least in *C. elegans*, a stimulated stress response can reduce the penetrance of partial loss-of-function mutations. We next tested whether the environmental stimulation of a stress response can have a similar effect. A mild environmental stimulus induces chaperone expression and promotes survival in subsequent environmental challenges, a response referred to as “hormesis” (21). We subjected animals to a transient heat shock as larvae to induce a stress response, allowed them to develop to adults, and examined the proportion of individuals affected by late-acting mutations. When a mutation was chaperone-dependent, a mild environmental challenge stimulated a reduction in penetrance (Fig. 1C). For example, an inactivating mutation in the zinc finger transcription factor LIN-29 caused abnormal male gonad migration in 46% of controls but only in 30% of animals receiving a prior heat stress ($P = 5 \times 10^{-4}$, a 35% reduction) (tables S3 and S4).

Beyond genetic differences, therefore, a second cause of variation in mutation outcome can be variation in the prior exposure of individuals to transient environmental stimuli (Fig. 1C and fig. S1, C to K). Although severe environmental stress can reduce mutation buffering (8, 9), transient environmental stimuli can promote it.

Isogenic individuals often show substantial variation in their response to a common environmental challenge (21–24). We tested whether this interindividual variation also affects the outcome of mutations. We applied a transient heat shock and sorted animals according to their induction of one of the stress-responsive reporters *hsp-16.2p::GFP* (green fluorescent protein)

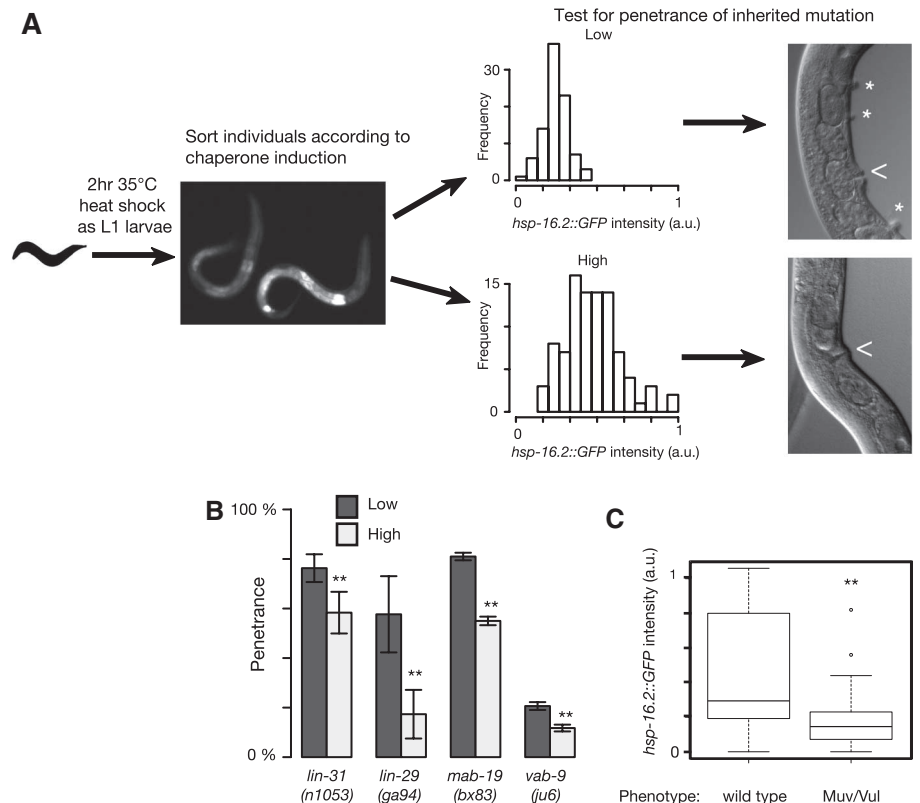


Fig. 2. Interindividual variation in a stress response predicts variation in mutation outcome. (A) Animals received a 2-hour 35°C heat shock as L1 larvae and were sorted 1 day later into “high” (right worm) and “low” (left worm) populations, according to the induction of an *hsp-16.2* chaperone promoter reporter. Histograms show representative GFP intensity distributions of sorted populations. Images on the right show the midbody region of *lin-31(n1053); hsp-16.2::GFP* animals. Asterisks mark pseudo-vulvae and arrowheads the vulva. (B) Mutation penetrance in the sorted populations for *lin-31(n1053); hsp-16.2::GFP*, *lin-29(ga94); hsp-16.2::GFP*, *mab-19(bx83); hsp-16.2::mcherry*, and *vab-9(ju6); hsp-16.2::GFP* (raw data in table S6). (C) GFP intensity in individual *lin-31(n1053); hsp-16.2::GFP* animals 12 hours after heat shock in larvae that did (right) and did not (left) ultimately develop an abnormal vulva phenotype. GFP expression levels are scaled between 0 and 1 in each panel.

or *hsp-16.2p::mcherry* (22). We found that animals in which a stronger stress response was induced had reduced mutation penetrance (Fig. 2,

A and B; fig. S2; and table S6). Quantifying the response in individual animals confirmed this finding: Animals that had a stronger stress

response were less likely to be affected by an inherited chaperone-dependent mutation (Fig. S2C and table S7).

Iso-genic individuals that induce higher chaperone levels are therefore more resistant to the effects of certain inherited mutations. These individuals are also more resistant to heat stress and live longer (22). Why, therefore, is a strong stress response not induced in all individuals in a population? Mutations that increase stress resistance can also have a pleiotropic effect on fitness: Perturbing the insulin-like signaling pathway in *C. elegans* can reduce fecundity (25), and increased chaperone expression in *Drosophila* can be detrimental (26). We reasoned, therefore, that a fitness trade-off might occur across isogenic individuals, with those having a stronger stress response also incurring a fitness cost.

Using the *hsp-16.2p::GFP* reporter to separate individuals after a heat stress, we observed that, although individuals that had a stronger response were more resistant to a subsequent severe heat stress (fig. S3 and table S8), they had a significantly reduced early fecundity (fig. S3D and table S9). If conditions remain benign, therefore, in-

dividuals that have a stronger stress response incur an important fitness cost (fig. S3D and table S9).

In addition to HSF-1, a second key regulator of the stress response is the FOXO transcription factor DAF-16. DAF-16 relocates from the cytoplasm to the nucleus after a heat stress and, with HSF-1, induces the expression of target genes, such as *hsp-16.2* (27, 28). Using a DAF-16::GFP fusion protein, we observed that the nuclear translocation of DAF-16 is rapid but that variation occurs across individuals in the time that DAF-16 remains in the nucleus (Fig. 3). For example, DAF-16 had been exported from the nucleus in half of animals ~100 min after a 3-hour heat shock (Fig. 3A). Sorting animals according to the nuclear residency time of DAF-16, we found that higher chaperone induction (fig. S4 and table S21), increased stress resistance (Fig. 3C, table S8), and reduced early fecundity (Fig. 3D and table S10) were all associated with prolonged DAF-16 signaling.

We next used a transcriptional reporter for an endogenously expressed chaperone, DAF-21 (Hsp90) to test whether variation in the stress response relates to preexisting variation in chap-

erone levels. Consistent with this idea, animals with higher *daf-21p::mcherry* expression before a heat shock developed greater thermotolerance (fig. S3G and table S8) and incurred a reproductive fitness cost after a mild heat stress (fig. S3H and table S11). Thus, interindividual variation in the response to a heat stress is, at least partially, due to preexisting molecular variation in a population.

We reasoned that this preexisting variation in chaperone expression might also affect the outcome of mutations. We used an RNA interference screen to define the individual chaperone dependence of different mutations: The results of this screen revealed that individual mutations differ in their chaperone dependence and provide a resource for future mechanistic work (fig. S5 and tables S13 to S20). In this screen, we identified the mutation *lin-31(n1053)* as strongly dependent on *daf-21* (Hsp90) activity (fig. S5D). We tested whether endogenous variation in *daf-21* expression predicted interindividual variation in the outcome of this mutation, by separating animals as larvae by their expression of the *daf-21p::mcherry* reporter and after the development of mutant phenotypes. Specifically for the *lin-31(n1053)* mutation, higher expression of the *daf-21* reporter was associated with a reduced mutation penetrance (Fig. 4 and table S22). This illustrates that even in the absence of a macroscopic environmental perturbation there can be predictable interindividual variation in the capacity to buffer mutations.

In summary, we have shown that, after a mild environmental stimulus, a trade-off occurs in *C. elegans* between the development of stress resistance and reproductive fitness. Preexisting

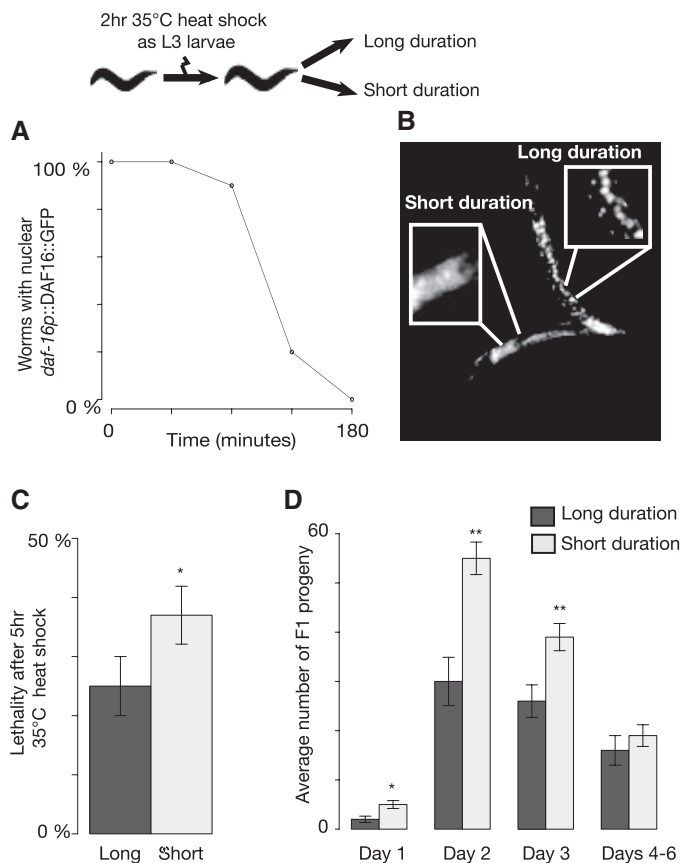


Fig. 3. A trade-off between the development of thermotolerance and reproductive fitness in an isogenic population. (A) Proportion of animals with nuclear DAF-16::GFP after a 2-hour 35°C heat stress at the L3 stage. (B) Two worms 50 min after heat shock: In the right individual ("long duration"), DAF-16::GFP is predominantly localized in the nucleus, whereas in the left ("short duration") individual, it is also in the cytoplasm. (C) Survival after a 5-hour heat shock at 35°C for the first (short duration) and last (long duration) 10 to 20% of individuals in which DAF-16::GFP returns to the cytoplasm. (D) The number of embryos laid as adults. * $P < 0.05$, ** $P < 0.01$, Fisher exact test for lethality, Wilcoxon rank sum test for fecundity (see also tables S8 and S10).

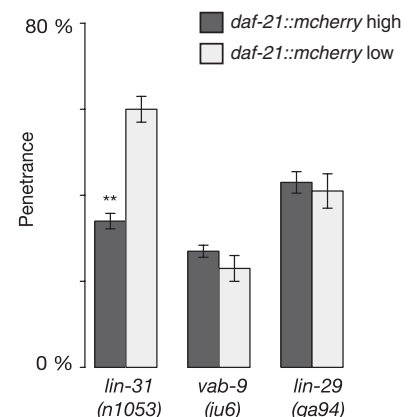


Fig. 4. Interindividual variation in the expression of an endogenous chaperone during larval development predicts mutation outcome. *lin-31(n1053); daf-21p::mcherry*, *lin-29(ga94); daf-21p::mcherry*, and *vab-9(ju6); daf-21p::mcherry* animals were sorted into high- and low-expressing populations as L4 larvae and scored for abnormal phenotypes as adults. Variation in the *daf-21* reporter predicts variation in the outcome of the *lin-31(n1053)* mutation. ** $P = 5.7 \times 10^{-4}$, Fisher exact test (table S22). Of these three mutations, only the penetrance of *lin-31(n1053)* is enhanced when *daf-21* is inhibited by RNA interference (fig. S5D).

molecular variation in an isogenic population means that, after an environmental challenge, stress signaling is prolonged in some individuals, and these individuals develop increased stress resistance but a lower reproductive potential. *C. elegans* is a naturally self-fertilizing species and, in the wild, is likely to experience highly variable environmental conditions. In a dynamic and unpredictable environment, the generation of such phenotypic diversity can be beneficial, acting as a “bet-hedging” strategy to diversify risk (29–33). We suggest that interindividual variation in stress signaling may therefore be beneficial, as it resolves a trade-off between the development of stress resistance and rapid reproduction.

We have also shown that an environmental stress response can stimulate genetic buffering and so protect individuals from inherited mutations. Variation in stress signaling, however, means that this induced capacity to buffer mutations differs predictably across individuals. The conservation of stress responses and their variability (21, 24) suggests that these concepts may apply quite widely, including perhaps in human genetic disease.

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Supporting Online Material

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Materials and Methods
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Molecular Mimicry Regulates ABA Signaling by SnRK2 Kinases and PP2C Phosphatases

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Absciscic acid (ABA) is an essential hormone for plants to survive environmental stresses. At the center of the ABA signaling network is a subfamily of type 2C protein phosphatases (PP2Cs), which form exclusive interactions with ABA receptors and subfamily 2 Snf1-related kinase (SnRK2s). Here, we report a SnRK2–PP2C complex structure, which reveals marked similarity in PP2C recognition by SnRK2 and ABA receptors. In the complex, the kinase activation loop docks into the active site of PP2C, while the conserved ABA-sensing tryptophan of PP2C inserts into the kinase catalytic cleft, thus mimicking receptor–PP2C interactions. These structural results provide a simple mechanism that directly couples ABA binding to SnRK2 kinase activation and highlight a new paradigm of kinase-phosphatase regulation through mutual packing of their catalytic sites.

Absciscic acid (ABA) is a vital plant hormone and a central regulator that protects plants against abiotic stresses such as drought and salinity. The core of the ABA signaling network comprises a subfamily of type 2C protein phosphatases (PP2Cs) and three Snf1-related kinases, SnRK2.2, 2.3, and 2.6 (1, 2), whose activities are tightly controlled by ABA. In the absence of ABA, SnRK2 kinases are in-

activated by PP2Cs, including ABI1, ABI2, and HAB1 (2–5), which physically interact with SnRK2s and dephosphorylate a serine residue in the kinase activation loop (S175 in SnRK2.6) whose phosphorylation is required for kinase activity (Fig. 1A) (6–8). ABA binding to the PYR/PYL/RCAR family of ABA receptors promotes the receptors to bind to the catalytic site of PP2Cs and inhibit their enzymatic activity

(Fig. 1A) (3–5, 9–12). In turn, ABA-induced inhibition of PP2Cs leads to SnRK2 activation by activation loop autophosphorylation (6, 7), which allows the SnRK2s to relay the ABA signal to downstream effectors (13, 14). In plants, SnRK2 activation loop phosphorylation may also involve unidentified upstream kinases (7, 15).

Recent structural studies have revealed a gate-latch-lock mechanism for PP2C inhibition by ABA receptors (9–12, 16). The ABA-binding pocket of receptors is flanked by two highly conserved loops that serve as a gate and latch.

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The gate in apo receptors is in open conformation. ABA binding induces the closure of the gate onto the latch and allows the receptor to dock into the PP2C active site, thus inhibiting PP2C activity by blocking substrate access. A conserved tryptophan of PP2C, which inserts between the gate and latch, functions as a molecular lock to further stabilize the receptor-PP2C complex (Fig. 1A). This tryptophan also functions as an ABA sensor through a water-mediated contact with the bound ABA in the receptor pocket. Thus, the gate of the receptors and the tryptophan lock of PP2Cs are both required for ABA-dependent receptor-PP2C interactions.

A fundamental but currently unresolved question is how SnRK2 activation is orchestrated with ABA binding and PP2C inhibition. To address this, we have solved the crystal structure of a SnRK2.6-HAB1 complex, which reveals a striking similarity between the SnRK2.6-HAB1 interface and the receptor-PP2C interface. The activation loop of SnRK2 is packed against the active site of PP2C in a manner mimicking the gate of ABA receptors. The conserved tryptophan of PP2Cs, which locks the gate of ABA receptors, inserts deeply into the kinase active cleft and completely blocks its activity. These shared structural elements for PP2C binding by ABA receptors and SnRK2 thus provide a direct mechanism that links ABA binding to kinase activation and highlights a new regulatory paradigm for phosphatase-kinase regulation through mutual packing of their catalytic sites.

SnRK2s contain a Snf1-related kinase domain and a highly acidic C-terminal segment termed ABA box, which is important for their interactions with PP2Cs (Fig. 1B and fig. S1) (6, 7). To characterize SnRK2-PP2C interactions, we purified the three SnRK2s (SnRK2.2, 2.3, and 2.6) and three PP2Cs (ABI1, ABI2, and HAB1). As determined by AlphaScreen (PerkinElmer) assays (fig. S2), each of the three SnRK2s was able to bind to the three PP2Cs with half maximal inhibitory concentration (IC_{50}) values of ~ 2 to $8 \mu M$, indicating a relatively weak binding between SnRK2s and PP2Cs (Fig. 1, C and D). The binding of the SnRK2.6 ABA box alone is 10- to 15-fold weaker compared with that of full-length SnRK2.6 (Fig. 1D), suggesting additional PP2C binding site(s) in SnRK2s.

To obtain the structure of a SnRK2-PP2C complex, we coexpressed and purified SnRK2s and PP2Cs in all nine combinations with an apparent 1:1 molar ratio, yet none of these complexes crystallized under any of the conditions tested. We hypothesized that the recalcitrance of these complexes to crystallization is due to their dynamic flexibility arising from the high dissociation rates associated with the low SnRK2-PP2C binding affinities. We therefore generated various SnRK2.6-HAB1 fusion proteins [SnRK2.6 is the strongest PP2C binder among the SnRK2s (Fig. 1C)] in which SnRK2.6 and HAB1 were separated by flexible linkers of various lengths (see Materials and Methods). A SnRK2.6-11 amino

acid linker-HAB1 fusion protein produced poorly diffracting crystals under a number of different conditions. Introduction of two adjacent surface-entropy-reduction mutations (Asp²⁹⁶→Ala²⁹⁶ and Glu²⁹⁷→Ala²⁹⁷, fig. S1) into the SnRK2.6 moiety greatly improved the quality of crystals and allowed us to solve the structure of the SnRK2.6-HAB1 complex at a resolution of $2.6 \text{ \AA}$ (structure statistics in table S1). Kinase activity was strongly inhibited in the fusion protein (fig. S3), and this inhibition was fully reversed in the presence of ABA-bound PYL2 receptor (fig. S3), indicating that both SnRK2.6 and HAB1 were active and functionally interacted with each other in the context of the fusion protein. Moreover, hydrogen/deuterium exchange (HDX) protection of this fusion protein was indistinguishable from that of a SnRK2.6-HAB1 complex formed with nonfused proteins (fig. S4), supporting that the fusion complex represents the functional complex formed by isolated proteins.

The overall structure of the SnRK2.6-HAB1 complex reveals a monomeric kinase-phosphatase complex with 1:1 stoichiometry (Fig. 2A). The salient feature of the SnRK2.6-HAB1 complex is the mutual packing of the kinase and phosphatase active sites, which form the major binding interface (Fig. 2A). SnRK2.6 contributes three separate regions within the kinase domain for binding to HAB1 (Fig. 2B). The first region is the SnRK2.6 activation loop, which is fully visible in the electron density map (fig. S5). This

activation loop inserts deeply into the catalytic cleft of HAB1 and thus mimics the gate loop of ABA receptors in receptor-PP2C complexes (Fig. 2 and fig. S6A) (3, 9, 10). In this orientation, S175 from the activation loop is directed toward the catalytic site of HAB1, which is consistent with the fact that pS175 of SnRK2.6 is preferentially dephosphorylated by HAB1 when compared with pS29 (fig. S7). The second region is the kinase catalytic cleft near residues Arg¹³⁹, Ile¹⁸³, and Glu¹⁴⁴ of SnRK2.6. This region mimics the cleft formed by the gate and latch regions of ABA receptors (3, 9, 10) and is occupied by the conserved ABA-sensing tryptophan [Trp³⁸⁵ (W385) in HAB1] (fig. S6B). The third region is the SnRK2.6 αG helix, which binds to the HAB1 loop region adjacent to the W385-containing HAB1 PYL-interaction site (Fig. 2B). The extensive interactions between the catalytic sites of kinase and phosphatase suggest that their activities have coevolved and are co-regulated.

The kinase domain-PP2C interface largely overlaps with the ABA receptor binding interface of HAB1 (Fig. 2, C and D, and fig. S6). The HAB1 structure in the SnRK2.6-PP2C complex is nearly identical to the HAB1 structure in the ABA receptor complex (root mean square deviation = $0.6 \text{ \AA}$ between the entire sets of Ca atoms of both HAB1 structures). We also solved the structures of the PP2C ABI2 in its apo state and as an ABI2-ABA-PYL2 complex (fig. S8). The PP2C components in these structures (apo PP2C, PP2C-

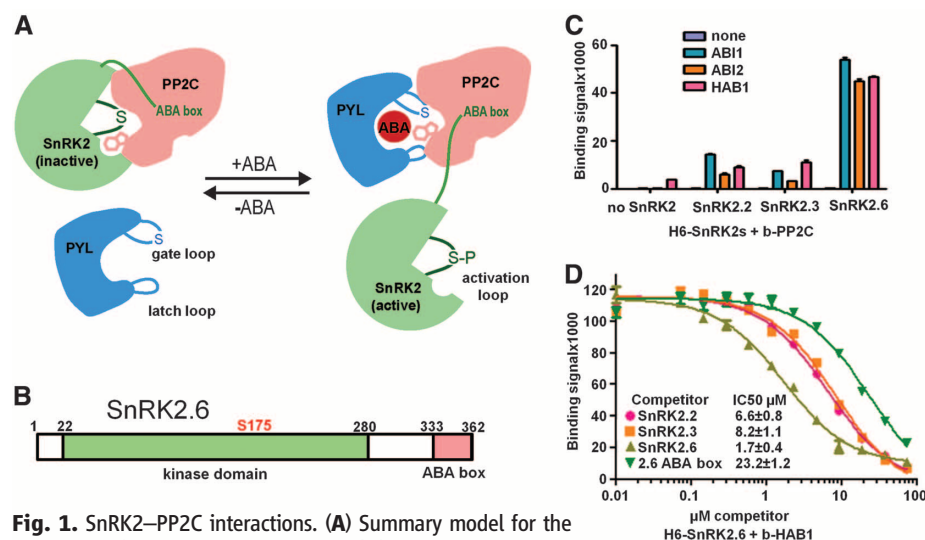


Fig. 1. SnRK2-PP2C interactions. (A) Summary model for the interactions between SnRK2, PP2C, and the ABA receptor PYR/PYL/RCAR in ABA signaling. In the absence of ABA, PP2C binds to the SnRK2 kinase domain and inhibits the kinase activity by dephosphorylating the activation loop serine and blocking the catalytic cleft. In the presence of ABA, ABA-receptor complex binds to PP2C and inhibits PP2C's catalytic activity by inserting the gate loop into the PP2C active cleft. PYL-mediated inhibition of PP2C allows activation of the kinase by activation loop autophosphorylation. The activated kinase then transmits the ABA signal by phosphorylating downstream factors. (B) Schematic presentation of the domain structure of SnRK2.6 with amino acid residue numbers shown on top. (C) Interactions of ABI1, ABI2, and HAB1 with SnRK2.2, 2.3, and 2.6. Binding of recombinant H6GST-SnRK2s to biotin-PP2Cs was determined by AlphaScreen assays (see fig. S2 and Materials and Methods). Error bars indicate SD ($n = 3$). (D) Inhibition of the interaction between H6GST-SnRK2.6 and biotin-HAB1 by untagged SnRK2s and SnRK2.6 ABA box peptide (amino acids 333 to 362). The IC_{50} values were derived from curve fitting. Error bars indicate SD ($n = 3$).

ABA-receptor, and PP2C-SnRK2) are all superimposable, indicating that PP2Cs have a fairly rigid fold (fig. S9). ABA-bound receptors and SnRK2s have thus adopted remarkably similar surface features that enable them to dock into the rigid PP2C scaffold (Fig. 2C). Consistently, the PYL2 ABA receptor-HAB1 interaction can be inhibited by increasing concentrations of the SnRK2.6 kinase domain (fig. S10). The shared use of HAB1 W385 and its catalytic site by ABA receptors and SnRK2.6 thus provides a direct mechanism that links ABA binding to kinase activation.

In addition to the kinase domain, SnRK2.6 interacts with PP2Cs via its highly acidic C-terminal ABA box (6, 7). However, we could not unambiguously resolve the ABA box. Consistent with its lack of clear electron density, the ABA box was not protected against HDX upon HAB1 binding (fig. S11). We hypothesized that this highly acidic ABA box (net charge: -14) should associate with a highly positive region of HAB1, which is at the proximity of the C terminus of SnRK2.6 (Fig. 3A and fig. S1). We therefore mutated all lysine and arginine residues within the positively charged HAB1 surface (Fig. 3A). Mutation of single residues within a tight cluster of six positively charged HAB1 surface amino acids disrupted interaction with the ABA box but had little effect on HAB1 catalytic activity (Fig. 3A and fig. S12), demonstrating that this region is critical for ABA box binding. On the other hand, alanine scanning mutagenesis of the SnRK2.6 ABA box identified many residues

important for HAB1 interaction, particularly the negatively charged residues (fig. S13). Together, these data suggest that ABA box-PP2C interactions are primarily mediated by charge interactions between the negatively charged ABA box and the positively charged PP2C surface. The ABA-box binding site in HAB1 is opposite of the kinase domain/ABA receptor binding site (Figs. 2A and 3A), consistent with the fact that ABA-box binding to PP2Cs is not affected by ABA or its receptors (fig. S14), further supporting the previous model that the ABA box provides a constant interaction for SnRK2.6 with PP2Cs (2).

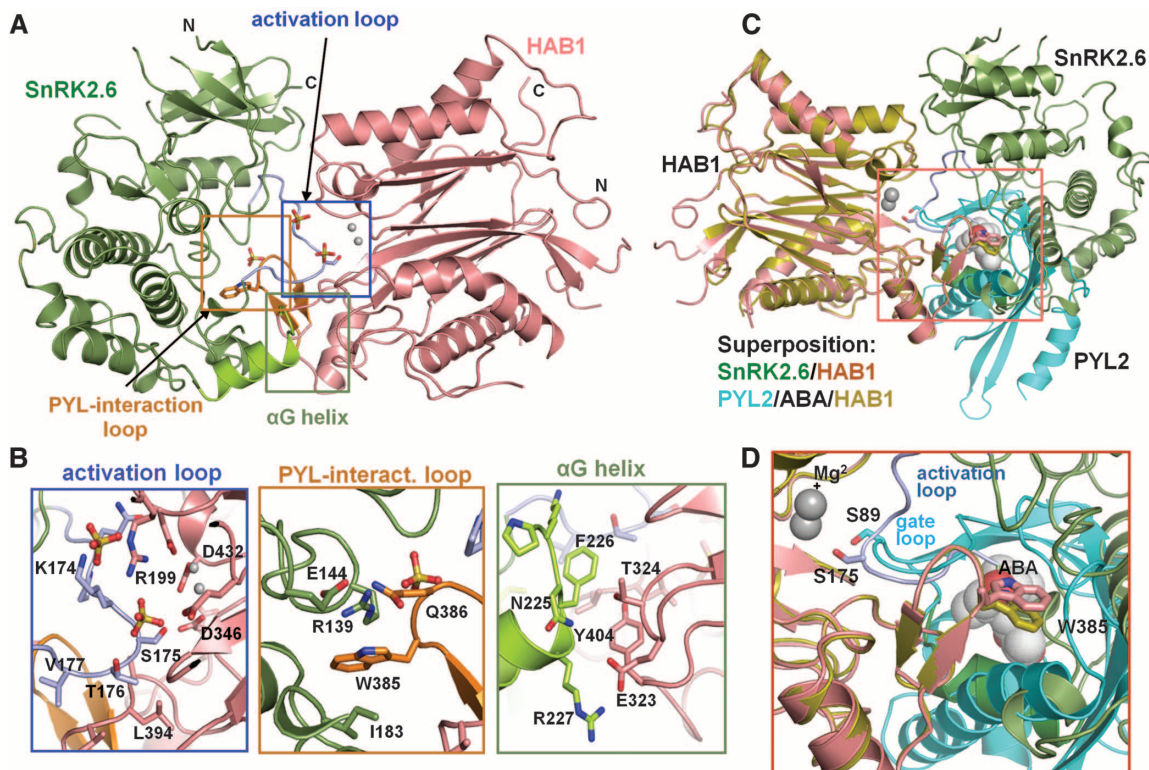
The separation of the ABA box binding site from the kinase domain/ABA receptor binding site in HAB1 suggests that the two sites function modularly. As shown in Fig. 3B, the Arg⁵⁰⁵→Ala⁵⁰⁵ (R505A) mutation in HAB1, which abolished the interaction between HAB1 and the ABA box (fig. S12), did not significantly weaken the interaction of HAB1 with full-length SnRK2.6. Similarly, mutations designed to disrupt each of the three kinase domain-interaction regions of HAB1 have little effect on the HAB1-SnRK2.6 interaction (Fig. 3B). In contrast, combination of the R505A mutation, which disrupted the ABA box interaction (fig. S12), with any mutation in the three kinase-interaction sites completely abolished SnRK2.6 binding (Fig. 3B). Together, these data further support that SnRK2.6 has two modular HAB1-binding sites.

The purified SnRK2.6 is intrinsically active as a kinase toward itself or its physiological sub-

strate ABF2 and can be inactivated by PP2C (Fig. 4A). The SnRK2.6-HAB1 structure suggests a two-step mechanism by which HAB1 completely inactivates SnRK2.6 (Fig. 4, A and B). The first step is mediated by the catalytic activity of HAB1, which dephosphorylates S175 from the activation loop, thus reducing SnRK2.6 activity to the basal level (Fig. 4C). The second step is the physical inhibition of the SnRK2.6 kinase domain by HAB1 (Fig. 4C). As shown in Fig. 2, insertion of HAB1 W385 into the SnRK2.6 catalytic cleft could physically block the access of substrate to the kinase active site, thus completely blocking the kinase activity. We have validated this two-step mechanism by a series of biochemical and mutational experiments (Fig. 4, A and B, and fig. S15). In these experiments, we show that catalytically inactive HAB1 is still capable of inhibiting SnRK2.6 (Fig. 4B and additional controls in fig. S15), in agreement with the observation that catalytic activities of PP2Cs are not required to inhibit SnRK2.6 activity (17, 18). Taken together, these data demonstrate that HAB1 inactivates SnRK2.6 by dephosphorylating S175 in the activation loop and by physically blocking the SnRK2.6 active site.

Our structural studies have revealed a molecular mimicry between ABA receptors and SnRK2 kinases with respect to how they bind to PP2Cs. Both SnRK2.6 and ABA receptors use a similar gate-and-lock mechanism to recognize PP2Cs. Given the large number of PP2Cs and SnRK2s encoded by plant genomes, of which only restricted sets are involved in ABA signaling, the

Fig. 2. Structures of the SnRK2.6-HAB1 complex. (A) A structure overview. SnRK2.6 is shown in green, the activation loop in light blue, and the α G helix in light green. HAB1 is colored pink, and its PYL2-interaction loop orange. The two catalytic Mg²⁺ ions are presented as gray spheres, and three sulfate ions as ball-stick models. (B) Details of the SnRK2.6-HAB1 interface with key residues shown in stick presentation. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; E, Glu; F, Phe; I, Ile; K, Lys; L, Leu; N, Asn; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (C and D) Overlay of the interaction surfaces in the SnRK2.6-HAB1 and PYL2-ABA-HAB1 complexes. SnRK2.6 S175 and HAB1 W385 are shown in stick presentation, ABA in ball presentation, and the HAB1 Mg²⁺ ions as spheres.



mutual recognition system of gate and lock likely ensures signal integrity by preventing the wrong players from being inappropriately regulated. The repeated use of gate-and-lock recognition as reported here thus provides a basis for the exquisite control of specificity in the ABA signaling network.

The packing interactions between the catalytic active sites of SnRK2.6 and HAB1 may also have important implications in other kinase-phosphatase signaling systems. PP2Cs are known to interact and regulate mammalian adenosine monophosphate-activated kinases (AMPK) (19). Furthermore, the recent discovery that the catalytically

inactive pseudophosphatases EGG-4 and EGG-5 recognize the activation loop of the autophosphorylating MBK-2 kinase and inhibit MBK-2 activity in the absence of any other protein (20, 21) suggests that kinase inactivation by phosphatase as observed in the SnRK2.6-HAB1 complex may represent a widespread mechanism for phosphatase-kinase regulation.

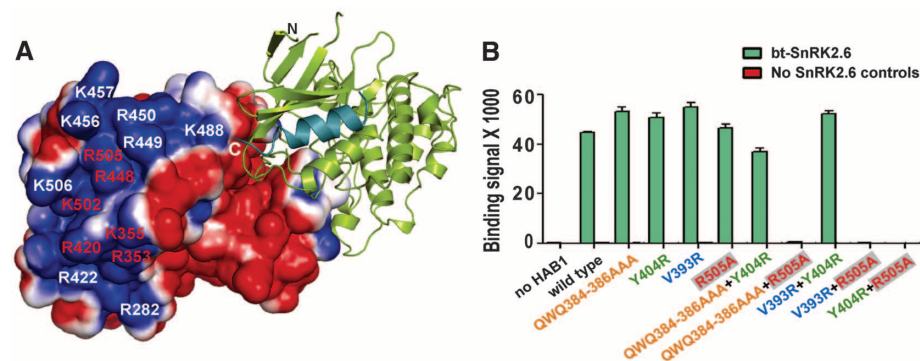


Fig. 3. SnRK2.6 and HAB1 associate with each other via two modular interactions. **(A)** Charge-distribution surface of HAB1 in the HAB1-SnRK2.6 complex with an electrostatic scale from -1 to $+1$ ev, corresponding to red and blue colors. The positively charged residues required for ABA box interaction are labeled in red. **(B)** Mutational analysis of the HAB1-SnRK2.6 interaction by AlphaScreen luminescence proximity assay. Error bars indicate SD ($n = 3$). HAB1 mutant proteins are color-coded on the basis of the mutated regions that bind to SnRK2.6. Q384A/W385A/Q386A, which is the “lock” that inserts into both SnRK2.6 and PYL2 ABA receptor, is colored in orange; V393R, which interacts with SnRK2.6 activation loop interaction, is in blue; Y404R, which binds to α G, is in green; R505A, which is required for ABA box interaction, is in red within gray boxes.

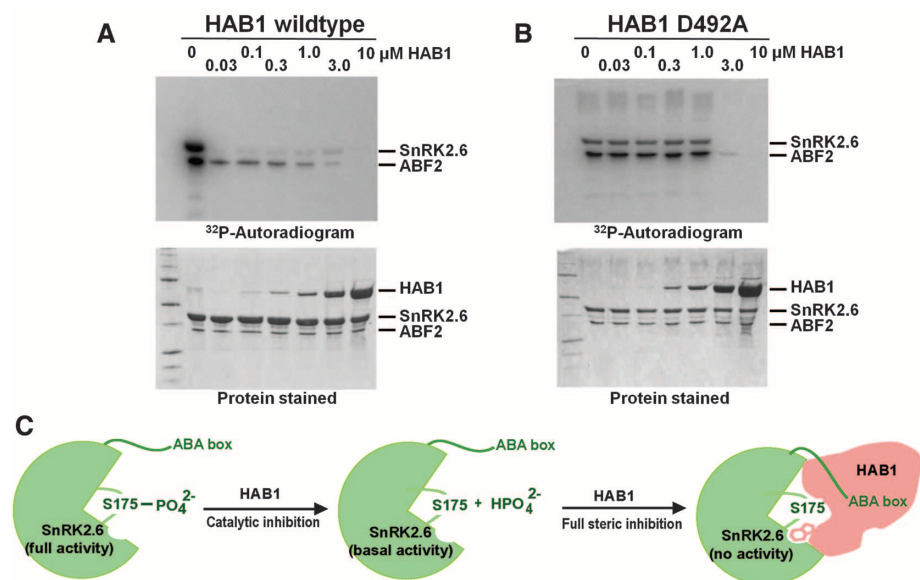


Fig. 4. Enzymatic and physical inhibition of SnRK2 activity by HAB1. **(A)** Inhibition of SnRK2.6 activity by HAB1. Increasing amounts of HAB1 were added to a kinase reaction with $5 \mu\text{M}$ SnRK2.6 and $1 \mu\text{M}$ of the SnRK2.6 substrate ABF2. At low concentrations, HAB1 inactivates SnRK2.6 catalytically ($>50\%$ kinase inhibition by HAB1 at a 100-fold molar excess of SnRK2.6). SnRK2.6 activity is only completely inhibited when HAB1 is at or above the molar amount of SnRK2.6. **(B)** Phosphatase activity of HAB1 is not required for its ability to inhibit SnRK2 kinase activity. Increasing amounts of the catalytically inactive HAB1 mutant D492A were added to a kinase reaction with constant concentrations of SnRK2.6 and ABF2. **(C)** Cartoon model for the two-step mechanism of inhibition of SnRK2.6 activity by HAB1. The first step is that HAB1 catalytically dephosphorylates S175 in the activation loop, thus reducing SnRK2.6 activity to the basal level. The second step is that at stoichiometric levels HAB1 physically inhibits SnRK2.6 activity by blocking its catalytic cleft.

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Supporting Online Material

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Equilibrative Nucleoside Transporter 3 Deficiency Perturbs Lysosome Function and Macrophage Homeostasis

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Lysosomal storage diseases (LSDs) are a group of heterogeneous disorders caused by defects in lysosomal enzymes or transporters, resulting in accumulation of undegraded macromolecules or metabolites. Macrophage numbers are expanded in several LSDs, leading to histiocytosis of unknown pathophysiology. Here, we found that mice lacking the equilibrative nucleoside transporter 3 (ENT3) developed a spontaneous and progressive macrophage-dominated histiocytosis. In the absence of ENT3, defective apoptotic cell clearance led to lysosomal nucleoside buildup, elevated intralysosomal pH, and altered macrophage function. The macrophage accumulation was partly due to increased macrophage colony-stimulating factor and receptor expression and signaling secondary to the lysosomal defects. These studies suggest a cellular and molecular basis for the development of histiocytosis in several human syndromes associated with ENT3 mutations and potentially other LSDs.

Hematopoietic cells lack de novo nucleoside generation and thus rely on membrane transporters to acquire nucleosides required for DNA and RNA synthesis, signal transduction, and adenosine 5'-triphosphate production. Transmembrane equilibrative and concentrative nucleoside transporters (ENTs and CNTs) carry nucleosides and cytotoxic nucleoside analogs across membranes (1, 2). The major source of nucleoside recycling is the degradation of nucleic acids, which is largely accomplished by histiocytes, the tissue macrophages. Macrophages are highly phagocytic cells of the immune system, which take up and degrade cellular debris within lysosomes (3). Although nucleoside uptake from the periphery by nucleoside transporters is well characterized (4), little is known about how nucleosides are exported from lysosomes where nucleic acid degradation occurs. In addition to substrate breakdown, lysosomes also participate in cellular function and metabolism (5). Patients with genetic defects in lysosomal hydrolyzing enzymes or transporters accumulate pathway metabolites that manifest in a variety of lysosomal storage diseases (LSDs) (6–8).

Because macrophages phagocytose and degrade the majority of dying cells and their nucleic acids, we hypothesized that a specific transporter (or transporters) equilibrating nucleosides out of lysosomes should be enriched in macrophages to

facilitate nucleoside recycling. A quantitative polymerase chain reaction survey of the ENTs in immune cells showed the highest expression of ENT3 in macrophages (fig. S1). To investigate the roles of ENT3 in nucleoside transport, we generated ENT3 knockout (ENT3^{−/−}) mice (9) (fig. S2).

ENT3^{−/−} mice developed spontaneous lymphadenopathy and splenomegaly by 8 weeks of age (Fig. 1, A and B) and had a significantly shorter life span compared to littermates (Fig. 1C). To investigate if any immune cell population was altered early in life, we examined 3-week-old animals and observed that the number of splenic macrophages was significantly increased in ENT3^{−/−} compared to littermates (Fig. 1D and fig. S3). In contrast, no differences in splenic T and B cell numbers were found. Thus, ENT3 deficiency affects macrophage development and/or proliferation. Macrophage infiltration was observed in multiple organs of ENT3^{−/−} mice, including spleen, liver, pancreas, intestine; exacerbated as animals aged (Fig. 1E and fig. S3); and progressed to histiocytic sarcoma in mice examined when moribund (fig. S4). To explore the mechanisms contributing to macrophage accumulation in ENT3^{−/−} mice, we examined macrophage apoptosis, proliferation, and cell surface activation markers. The frequency of macrophages that stained positive for TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling) was similar between wild-type and ENT3^{−/−} mice, with increased absolute numbers of TUNEL⁺ ENT3^{−/−} macrophages (Fig. 1F). Cycling 5-bromo-2'-deoxyuridine-positive (BrdU⁺) splenic macrophages were significantly increased in both frequency and numbers, indicating active cycling as well as extramedullary myelopoiesis in ENT3^{−/−} mice (Fig. 1G and fig. S5). However, ENT3^{−/−} mice had normal basal concentrations of inflammatory serum cytokines and their splenic ma-

crophages had normal surface activation markers (figs. S6 and S7), suggesting that myelopoiesis was not caused by an acute inflammatory response. Furthermore, macrophage expansion was recapitulated in normal irradiated recipients transplanted with ENT3^{−/−} bone marrow cells, suggesting that hematopoietic cell defects contributed to the enhanced myelopoiesis (Fig. 1H and fig. S3D).

We hypothesized that ENT3 is the nucleoside transporter crucial for lysosomal nucleoside export and its absence may affect the late endosomal-lysosomal system. ENT3^{−/−} splenic macrophages had increased numbers of lysosomal-associated membrane protein 1 (lamp-1)⁺ vesicles (Fig. 2A and fig. S8). Furthermore, electron microscopy (Fig. 2A) showed that ENT3^{−/−} macrophage lysosomes were enlarged in size and contained undigested materials (Fig. 2B), accompanied with elevated intralysosomal pH (Fig. 2C). Thus, ENT3 deficiency causes lysosomal abnormalities in macrophages. To dissociate in vivo macrophage developmental abnormalities caused by ENT3 deficiency from functional defects, we used bone marrow-derived macrophage (BMDM) cultures for phenotypic and functional analyses. ENT3^{−/−} and wild-type BMDMs had comparable surface marker phenotypes and lipopolysaccharide-induced cytokine responses, suggesting that ENT3^{−/−} BMDMs were developmentally normal with respect to these parameters (fig. S9). We then examined phagocytosis, phagosome-lysosome fusion, and degradation of engulfed materials in BMDMs. By tracking fluorescently labeled bacteria, we found no significant impairment of phagocytosis or phagosome-lysosome fusion (fig. S10) in ENT3^{−/−} BMDMs. However, when the BMDMs were incubated with apoptotic thymocytes or challenged with live bacteria to mimic in vivo macrophage functions, ENT3^{−/−} BMDMs showed both delayed degradation of apoptotic thymocytes (Fig. 2D and fig. S10D) and bacterial killing (Fig. 2E), implying defects in lysosomal functions. To examine the in vivo functional consequences of ENT3^{−/−} macrophage lysosomal perturbations, we challenged mice with lethal doses of *Listeria monocytogenes*. ENT3^{−/−} animals succumbed to *L. monocytogenes* infection significantly earlier compared to wild-type littermates (Fig. 2F). In addition, pathogen load was significantly increased in spleen, liver, and kidney of infected ENT3^{−/−} mice (fig. S10E). Thus, ENT3^{−/−} macrophages appear to have dysfunctional lysosomes incapable of normal cellular functions and overall defense.

The observations of ENT3^{−/−} macrophage lysosomal abnormalities prompted us to hypothesize that perturbed nucleoside trafficking causes lysosomal defects in ENT3^{−/−} macrophages, as seen in other LSDs (10, 11). Adenosine, a representative nucleoside, accumulated in whole-cell extracts and purified lysosomes derived from ex vivo splenic ENT3^{−/−} macrophages (Fig. 3, A and B), suggesting that lack of ENT3 impairs lysosomal nucleoside export. To examine the cause of this phenomenon in ENT3^{−/−} macrophages, we performed kinetic studies in BMDMs. Adenosine

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concentrations were similar between ENT3^{-/-} and littermate BMDMs before stimulation; however, upon challenge with exogenous apoptotic thymocytes, accumulation of adenosine was observed in ENT3^{-/-} BMDM whole-cell extracts (Fig. 3C) and purified lysosomal fraction (fig. S11). Intracellular nucleoside homeostasis could be restored

in ENT3^{-/-} BMDMs retrovirally reconstituted with wild-type mouse and human ENT3 (Fig. 3, D and E), but not by the human ENT3 mutant 427S identified in H syndrome patients (12) (Fig. 3E). Excessive lysosomal nucleosides could compete for free protons within the lysosome and lead to their alkalization (13). Indeed, coincident with

adenosine accumulation, ENT3^{-/-} BMDMs as well as human ENT3-mutant 427S-expressing BMDMs challenged with apoptotic thymocytes had elevated intralysosomal pH (Fig. 3F and fig. S12). Thus, in the absence of ENT3, excess accumulation of nucleosides in lysosomes led to perturbed lysosomal functions and homeostasis of macrophages.

Fig. 1. ENT3 deficiency leads to histiocytosis. Photographs of 8-week-old inguinal and mesenteric lymph nodes (A) and spleen (B). (C) Life span of ENT3^{-/-} and wild-type littermates ($n = 30$). (D) Splenic cellularity of 3-week-old mice ($n = 4$). (E) Spleen immunohistochemistry (F4/80 antibody staining). (F) Splenic macrophages undergoing apoptosis were analyzed by flow-cytometry (left) and quantified (right). (G) Proliferation of splenic macrophages was examined by flow cytometry (left) and quantified (right) ($n = 5$). (H) Splenic macrophages in radiation bone marrow chimeras of ENT3^{-/-} and wild-type littermates ($n = 3$ to 5). $**P < 0.05$, $***P < 0.001$ (unpaired two-tailed t test); n.s., no significant difference. Error bars represent SEM.

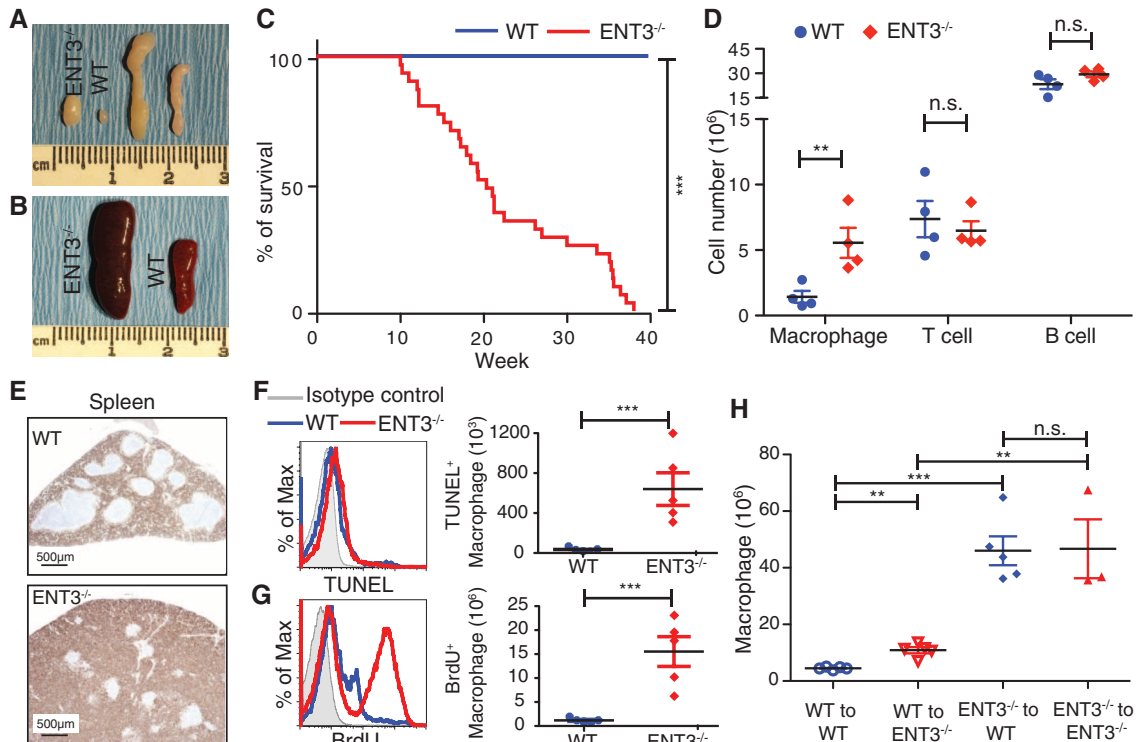
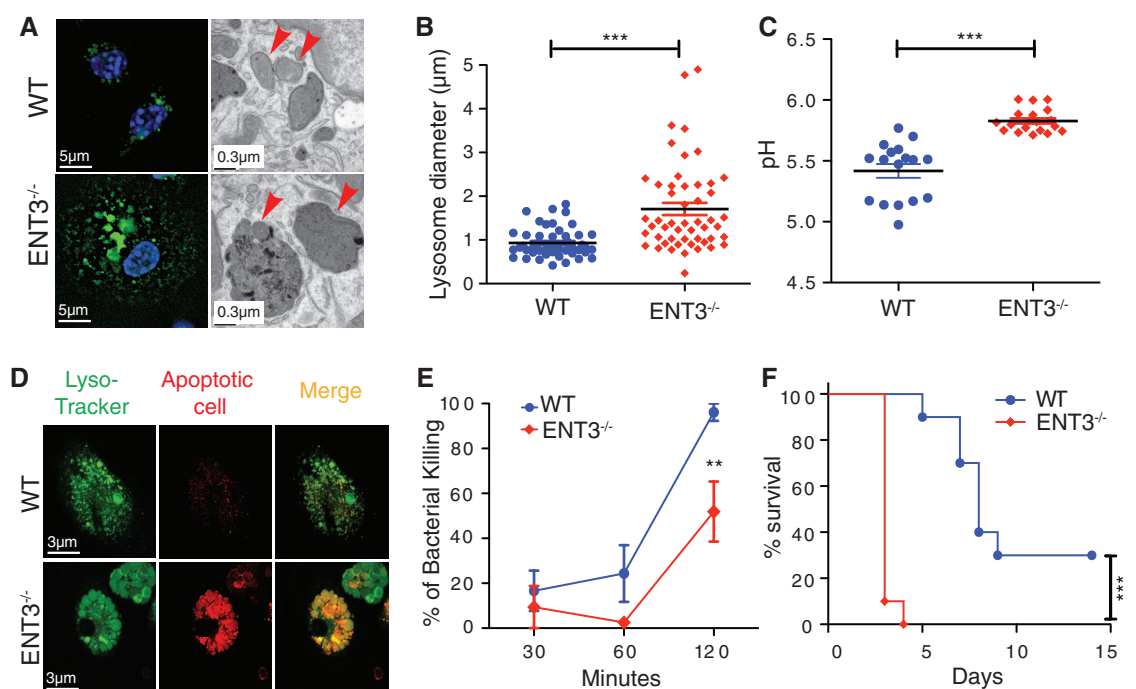


Fig. 2. ENT3 deficiency causes morphological and functional changes in macrophages. (A) (Left) Ex vivo splenic macrophages show increased lamp1 expression (green) and (right) electron microscopy showing undigested materials in ENT3^{-/-} splenic macrophage lysosomes (red arrowheads). (B) Lysosome diameters. (C) Intralysosomal pH in splenic macrophages ($n = 17$ individual cells; representative of three independent experiments). (D) Processing of engulfed apoptotic thymocytes in BMDMs (24 hours). (E) In vitro bacterial killing assay of *Escherichia coli* with BMDMs. (F) *Listeria monocytogenes*-induced mortality (2×10^6 CFU, given intravenously) in wild-type littermates and ENT3^{-/-} animals ($n = 10$). Statistics were done by unpaired t test or Mantel-Cox test. $**P < 0.05$, $***P < 0.001$; n.s., no significant difference. Error bars represent SEM.



Next, we investigated pathways that could drive $ENT3^{-/-}$ myeloproliferation and looked for perturbations in macrophage growth factor levels or their cognate receptors. Surface expression of macrophage colony-stimulating factor receptor (M-CSFR) was significantly higher in $ENT3^{-/-}$ splenic macrophages (Fig. 4A), whereas levels of the β -chain subunit of granulocyte-macrophage colony-stimulating factor (GM-CSFR) and fms-like tyrosine kinase receptor-3 (Flt3) were comparable to those of wild-type macrophages (fig. S13). In addition, $ENT3^{-/-}$ mice had elevated serum concentrations of M-CSF, although G-CSF, Flt3L, and IL-34 serum concentrations were normal (Fig. 4B and fig. S13). The high levels of M-CSF and its cognate receptor were accompanied by increased signaling through M-CSFR indicated by the phosphorylation status of Y723 of M-CSFR (14) in $ENT3^{-/-}$ macrophages (fig. S14). The higher M-CSF concentration was not due to increased production of M-CSF by $ENT3^{-/-}$ splenic and bone marrow stromal cells or macrophages (fig. S15), but more likely a result of defective M-CSF clearance (fig. S16). M-CSFR transcription was also similar between splenic $ENT3^{-/-}$ and wild-type macrophages (fig. S17), suggesting a posttranslational alteration of M-CSFR in $ENT3^{-/-}$ mice.

To assess the contribution of elevated M-CSF/M-CSFR axis to the accumulation of macrophages in $ENT3^{-/-}$ mice, we blocked this pathway using a neutralizing antibody against M-CSF (anti-M-CSF). After 4 or 24 weeks of treatment, $ENT3^{-/-}$ mice had 50% fewer splenic macrophages compared to the isotype control antibody-treated group (Fig. 4C and figs. S18 and S19). Anti-M-CSF treatment in $ENT3^{-/-}$ mice also resulted in lower granulocyte and macrophage CFU numbers but not Ki67⁺

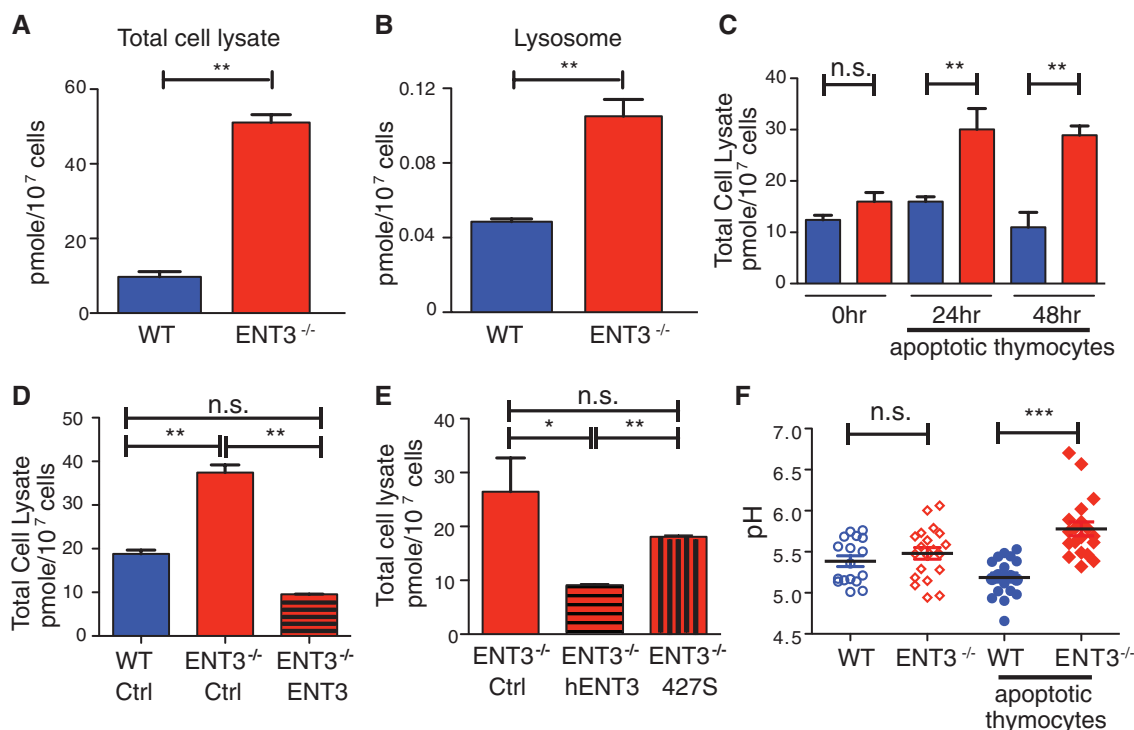
cycling splenic macrophages (figs. S18 and S19), suggesting that the M-CSF/M-CSFR axis participates in myeloproliferation primarily through enhanced extramedullary myelopoiesis. In addition, $ENT3^{-/-}$ splenic macrophages had elevated and sustained M-CSFR expression even after anti-M-CSF treatment (fig. S18), supporting the hypothesis that $ENT3^{-/-}$ macrophages have a posttranslational modification of M-CSFR that is independent of ligand engagement. M-CSF blockade did not alter the mortality seen in $ENT3^{-/-}$ mice (fig. S19), suggesting that the residual histiocytosis and mortality involve factors beyond surveyed myeloid growth factors (fig. S13).

To understand why M-CSF blockade rescued only partially the $ENT3^{-/-}$ histiocytosis phenotype, we investigated factors that may be affected by impaired macrophage functions and found accumulation of apoptotic cells in the spleen after anti-M-CSF treatment (fig. S19). Because exogenous apoptotic cell burden induces lysosomal dysfunctions in the $ENT3^{-/-}$ macrophages (Fig. 3, C and F), we asked if its removal could prevent histiocytosis. In mixed bone marrow chimeras, the presence of wild-type bone marrow cells alleviated the macrophage expansion phenotype and removed apoptotic cells in a dose-dependent manner (Fig. 4D and fig. S20). Furthermore, the M-CSFR surface expression on $ENT3^{-/-}$ macrophages reverted to normal in the presence of $ENT3^{-/-}$ competent cells (Fig. 4E). To examine whether the exogenous nucleic acid pressure directly affects M-CSFR turnover, we stressed BMDMs with apoptotic thymocytes and found that surface expression of M-CSFR was increased in $ENT3^{-/-}$ compared with wild-type BMDMs (Fig. 4F and fig. S21). Moreover, ligand-induced M-CSFR en-

docytoysis appear similar between wild-type and $ENT3^{-/-}$ BMDMs (fig. S22). Thus, the dysregulation of M-CSFR turnover in $ENT3^{-/-}$ cells is ligand-independent and a consequence of exogenous nucleic acid pressure. Because the down-regulation of M-CSFR and termination of M-CSF/M-CSFR signaling rely ultimately on lysosomal degradation (15, 16), we examined whether lysosomal alkalinization can explain the M-CSFR dysregulation in $ENT3^{-/-}$ animals. By treating wild-type BMDM with bafilomycin, a lysosomal pH-altering agent, we found that lysosomal alkalinization leads to higher surface expression and less degradation of M-CSFR (Fig. 4, G and H). These data provide a causative link between lysosomal nucleoside accumulation and alkalinization to dysregulated M-CSFR turnover and signaling.

Here, we have identified $ENT3$ as an essential transmembrane transporter to maintain lysosome integrity by regulating nucleoside trafficking. $ENT3$ deficiency, through defects in the lysosomal system, caused ineffective apoptotic cell clearance and increased M-CSF signaling that contribute to elevated numbers of macrophages and histiocytosis. Loss-of-function human $ENT3$ mutants have recently been associated with familial Rosai-Dorfman disease, Faisalabad histiocytosis, H syndrome, and pigmented hypertrichosis with insulin-dependent diabetes (12, 17–21), diseases that in some cases have lymphadenopathy, splenomegaly, and increased infiltration of macrophages in various immune and nonimmune organs. Our findings provide the cellular and molecular mechanism for the histiocytosis seen in these patients and potentially other LSDs. We can envisage that this mechanism, resulting in impaired dead cell removal and M-CSF/M-CSFR dysfunction

Fig. 3. Lysosomal nucleoside accumulation and pH increase in $ENT3^{-/-}$ macrophages. Intracellular adenosine concentrations from splenic macrophage whole-cell lysate (A) or enriched lysosomes (B) ($n = 3$). (C) Intracellular adenosine concentrations in BMDMs challenged with apoptotic thymocytes. (D and E) Intracellular adenosine in retrovirally transduced BMDMs after incubation with apoptotic thymocytes. (F) Intralysosomal pH values of BMDMs at 48 hours after incubation with apoptotic thymocytes. Data in (A to C) and (D to F) are representative of three and two independent experiments, respectively. ** $P < 0.05$, *** $P < 0.001$; n.s., no significant difference. Error bars represent SEM.



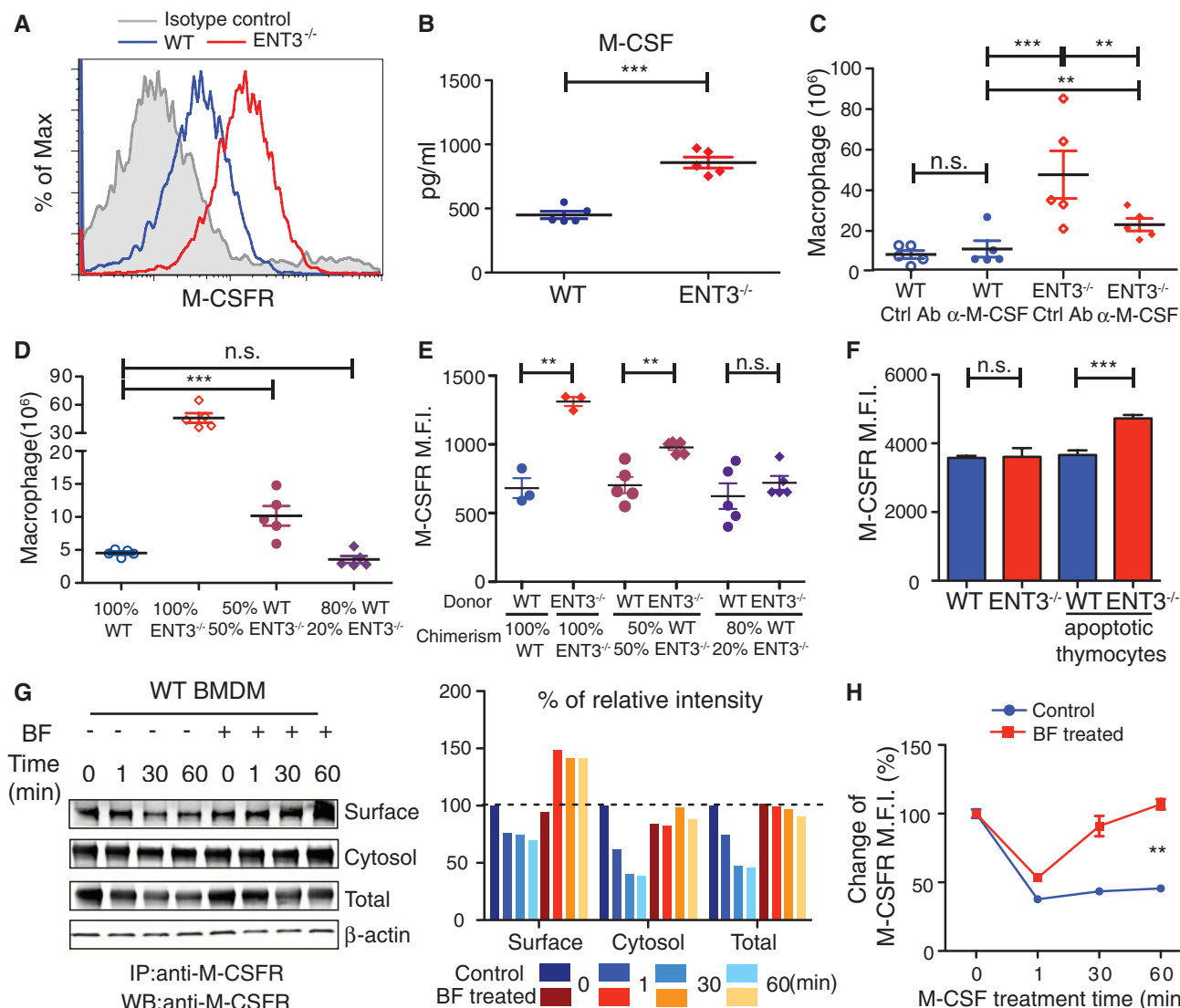


Fig. 4. Altered M-CSF/M-CSF receptor axis contributes to the myeloproliferative phenotype in $ENT3^{-/-}$ animals. **(A)** Surface expression of M-CSFR on splenic macrophages. **(B)** Serum concentrations of M-CSF (12 weeks, $n = 5$). **(C)** Splenic macrophage numbers after 4 weeks of treatment with neutralizing anti-M-CSF or control antibodies ($n = 5$). Number of splenic macrophages **(D)** and quantification of surface M-CSFR **(E)** in wild-type- $ENT3^{-/-}$ mixed bone marrow chimera mice ($n = 3$ to 5 per group). Decrease in average M-CSFR

surface expression occurs in $ENT3^{-/-}$ macrophages. **(F)** M-CSFR surface expression on BMDMs upon apoptotic thymocyte challenge (72 hours, $n = 3$). **(G)** Immunoblot analysis (left) and quantification (right) of M-CSFR turnover on wild-type BMDMs after bafilomycin treatment. **(H)** Flow cytometric analysis of surface M-CSFR turnover with bafilomycin treatment ($n = 3$). P values were determined by unpaired t test. $^{**}P < 0.05$, $^{***}P < 0.001$; n.s., no significant difference. Error bars represent SEM.

and histiocytosis, may be contributing pathways for a broader range of lysosomal storage diseases, independent of $ENT3$ mutations.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1213682/DC1
Materials and Methods
Figs. S1 to S24
References

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Dystroglycan Function Requires Xylosyl- and Glucuronyltransferase Activities of LARGE

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Posttranslational modification of alpha-dystroglycan (α -DG) by the like-acetylglucosaminyltransferase (LARGE) is required for it to function as an extracellular matrix (ECM) receptor. Mutations in the *LARGE* gene have been identified in congenital muscular dystrophy patients with brain abnormalities. However, the precise function of LARGE remains unclear. Here we found that LARGE could act as a bifunctional glycosyltransferase, with both xylosyltransferase and glucuronyltransferase activities, which produced repeating units of [–3-xylose– α 1,3-glucuronic acid– β 1–]. This modification allowed α -DG to bind laminin-G domain–containing ECM ligands.

Protein glycosylation is important in determining cellular localization, modulating structural conformation, and providing recognition sites for other molecules (1). Dystroglycan (DG) is a highly glycosylated basement membrane receptor involved in a variety of physiological processes that maintain skeletal muscle

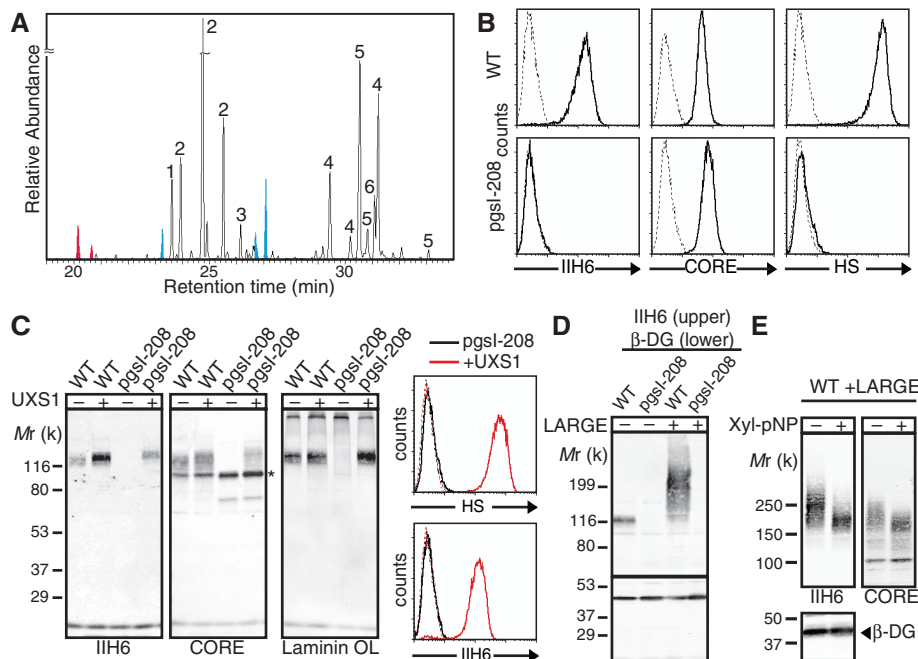
membrane integrity, as well as central nervous system structure and function; it also serves as a receptor for Old World arenaviruses. Composed of a cell surface α subunit and a transmembrane β subunit (2), α -DG acts as a receptor for laminin-G domain–containing extracellular matrix (ECM) proteins such as laminin, agrin, and neuroligin (3). α -DG undergoes *N*-glycosylation, mucin-type *O*-glycosylation, and *O*-mannosylation, and a yet-to-be identified modification of a phosphorylated *O*-mannosyl glycan is believed to be responsible for ligand binding (4). Perturbed glycosylation can lead to reduced ligand binding by α -DG and is a common pathologic feature among several congenital and limb-girdle muscular dystrophies. Mutations in the *LARGE* gene (5) and several others involved in *O*-mannosyl glycan synthesis have been identified in these disorders (6). The overexpression of LARGE enhances functional

modification of α -DG, as well as laminin binding in cultured cells (7). Moreover, it circumvents defects in the modification of α -DG in cells from patients with several genetically distinct types of congenital muscular dystrophies (8). Although LARGE has been implicated in the postphosphorylation modification pathway that assembles the laminin-binding moiety onto the phosphorylated *O*-mannose of α -DG, its molecular role and the laminin binding epitope remain to be identified.

We investigated the LARGE-dependent modification on α -DG by performing compositional sugar analysis of the recombinant α -DG produced in LARGE-expressing human embryonic kidney (HEK) 293 cells (fig. S1). Samples were treated with glycosidases to eliminate glycans unrelated to the laminin-binding moiety (4). We detected: (i) known compositional sugars of the α -DG *O*-mannosyl glycan—specifically *N*-acetylglucosamine (GlcNAc), galactose (Gal), *N*-acetylgalactosamine (GalNAc), and mannose (Man) (4); (ii) glucose (Glc), which was likely a contaminant; and (iii) substantial amounts of glucuronic acid (GlcA) and xylose (Xyl) (Fig. 1A). GlcA and Xyl are essential components of heparan sulfate (HS) and chondroitin-dermatan sulfate (CS-DS) glycosaminoglycans (GAGs), whose biosynthesis is initiated by linkage of tetrasaccharide GlcA β 1-3Gal β 1-3Gal β 1-4Xyl β 1- to proteoglycan core proteins. Notably, GAGs are not required for α -DG to bind laminin (9, 10) (fig. S2).

We tested whether xylosylation unrelated to GAG formation is involved in functional modification of α -DG by assessing uridine 5'-diphosphate (UDP)-xylose synthase 1 (UXS1)-deficient Chinese hamster ovary (CHO) cells (line pgsI-208), which are deficient in GAG synthesis because they

Fig. 1. Xylosylation is required for functional modification of α -DG. **(A)** Compositional sugar analysis by gas chromatography-MS with trimethylsilyl derivatization using α -DG produced by LARGE-overexpressing HEK293 cells digested with peptide: *N*-glycosidase F, *O*-glycosidase, and neuraminidase. Peaks shown in red represent Xyl, those in blue represent GlcA, and numbers indicate 1, Man; 2, Gal; 3, Glc; 4, GlcNAc; 5, GalNAc; and 6, inositol (internal control). Some of the detected Gal was derived from the Jacalin elution buffer we used for the purification. **(B)** Flow cytometry of WT or UXS1-deficient (pgsI-208) CHO cells surface stained with antibodies against the laminin-binding epitope of α -DG (IIH6), the α -DG core protein (CORE), or heparan sulfate (HS). Dashed line, secondary antibody alone. **(C)** Functional modification of α -DG in cells overexpressing UXS1. (Left) Immunoblotting or laminin overlay (OL) assays of glycoproteins. *Mr*, relative molecular mass. Asterisk indicates nonfunctional α -DG that appeared as a sharp band because of hypomodification by CORE staining. (Right) Flow cytometry for surface staining with HS or IIH6. **(D)** Immunoblotting for IIH6 and β -DG-specific antibody reactivity in WT and pgsI-208 cells with or without overexpression of LARGE. **(E)** Immunoblotting for IIH6, CORE, or β -DG-specific antibody reactivity in LARGE-expressing WT cells treated with or without Xyl- α -pNP.



lack UDP-Xyl (11). Immunoblotting with IIH6 (an antibody that recognizes the laminin-binding form of α -DG), laminin overlay of wheat germ agglutinin-enriched proteins (glycoproteins), and flow cytometric analysis revealed that these cells were defective in synthesis of the functional modification of α -DG (Fig. 1, B and C). Ectopic expression of UXS1 in pgsI-208 cells rescued HS-GAG synthesis, as well as the IIH6 reactivity and laminin-binding ability of α -DG (Fig. 1C). In contrast, overexpression of LARGE in these cells did not overcome the defect (Fig. 1D). Thus, xylosylation may be required for formation of the acceptor substrate on which LARGE acts, and/or LARGE itself may be involved in the transfer of Xyl to α -DG. Xylosides with hydrophobic moieties that help penetrate lipid bilayers are widely used to inhibit certain glycosyltransferases acting on Xyl, because they serve as artificial acceptors (12). Given that one of the two domains of LARGE belongs to glycosyltransferase family 8 (GT8), whose members generate products with an α -linked glycosidic bond (13), we tested α -D-xyloside for the ability to inhibit functional modification of α -DG. Indeed, treat-

ment of LARGE-expressing CHO cells with *p*-nitrophenyl- α -D-xyloside (Xyl- α -pNP) reduced α -DG glycosylation (Fig. 1E). Thus xyloside may compete against endogenous α -DG bearing an α -linked Xyl for LARGE-dependent modification.

Next, we examined the enzymatic activity of LARGE with respect to Xyl- α -pNP as the acceptor, using a secreted form of LARGE that lacks its transmembrane domain (LARGETM). Purified LARGETM (fig. S3) was incubated with Xyl- α -pNP and each donor substrate, and the reaction products were separated by high-performance liquid chromatography (HPLC). A unique peak was detected only when UDP-GlcA was used as the donor (Fig. 2A for UDP-GlcA and fig. S4 for other donors). This activity was specific to α -linked Xyl (fig. S5). We purified the product (fig. S6) and analyzed it by mass spectrometry (MS). The MS/MS fragmentation pattern of a parent ion at mass/charge ratio, *m/z*, of 446.25 [M-H]⁻ demonstrated that GlcA was attached to Xyl- α -pNP (Fig. 2B and fig. S7). Because LARGE showed glucuronyltransferase (GlcA-T) activity toward α -linked Xyl and has two glycosyltransferase-like domains, we hy-

pothesized that it could also act on a nonreducing terminal GlcA. When 4-methylumbelliferyl- β -D-glucuronide (GlcA- β -MU) was used as an acceptor, a unique peak was observed only with UDP-Xyl (Fig. 2C for UDP-Xyl and fig. S8 for other donors); the attachment of Xyl to GlcA- β -MU was confirmed by MS (Fig. 2D and fig. S9).

Because mutations in the LARGE Asp-X-Asp (DXD; X, any amino acid) motifs (Fig. 2E), which are highly conserved across glycosyltransferases, lead to failed functional modification of α -DG (14), we generated constructs with such mutations for further testing of LARGE glycosyltransferase activity. In the case of the DXD1 mutant in which Asn replaces Asp at two sites (D242N/D244N), GlcA-T activity was comparable to that in wild type (WT), whereas Xyl-T activity was undetectable (Fig. 2F). In the DXD3 mutant D563N/D565N, in contrast, Xyl-T activity, but not GlcA-T activity, was present (Fig. 2F). Thus, LARGE is a bifunctional glycosyltransferase composed of two distinct catalytic domains, Xyl-T and GlcA-T.

Negatively charged oligosaccharides likely contribute to binding between α -DG and laminin

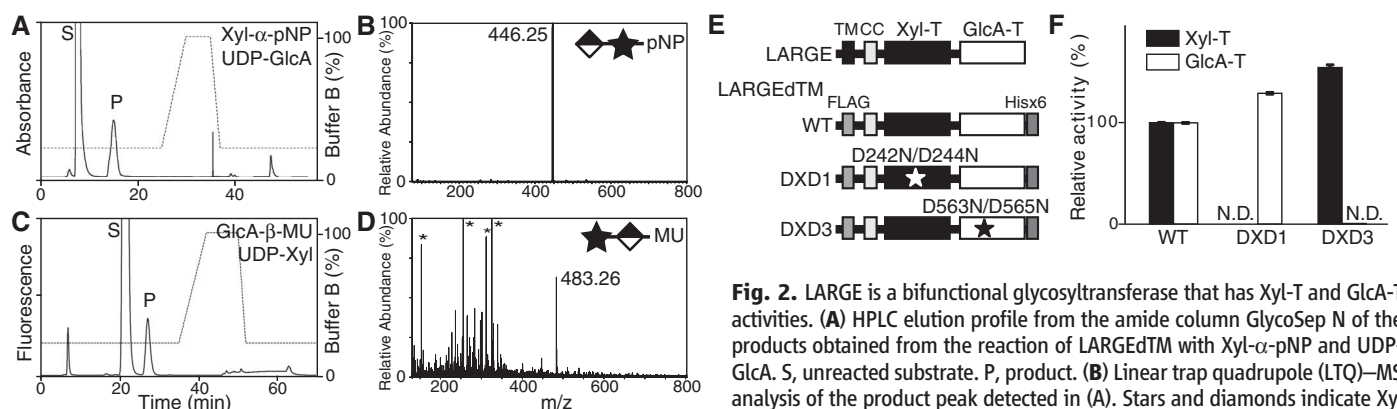


Fig. 2. LARGE is a bifunctional glycosyltransferase that has Xyl-T and GlcA-T activities. (A) HPLC elution profile from the amide column GlycoSep N of the products obtained from the reaction of LARGETM with Xyl- α -pNP and UDP-GlcA. S, unreacted substrate. P, product. (B) Linear trap quadrupole (LTQ)-MS analysis of the product peak detected in (A). Stars and diamonds indicate Xyl and GlcA, respectively. MS/MS fragmentation pattern (fig. S7) confirmed that

the ion with *m/z* of 446.3 [M-H]⁻ is Xyl- α -pNP with an added GlcA. (C) HPLC elution profile from GlycoSep N of the products obtained from the reaction of LARGETM with GlcA- β -MU and UDP-Xyl. (D) As in the legend to (B), for the product isolated from the reaction analyzed in (C). The MS/MS fragmentation pattern (fig. S9) confirmed that the ion with *m/z* of 483.3 [M-H]⁻ is GlcA- β -MU with an added Xyl. Asterisks indicate background ions. (E) Schematic representation of the WT and DXD mutants of LARGETM constructs used in the assay. The locations of the mutations in the DXD motifs are symbolized by stars. (F) GlcA-T and Xyl-T activities of WT and mutant LARGETMs. Relative activity (%) with respect to WT, and the standard deviation in triplicate experiments, are shown. TM, transmembrane domain. CC, coiled-coil domain. N.D., not detected.

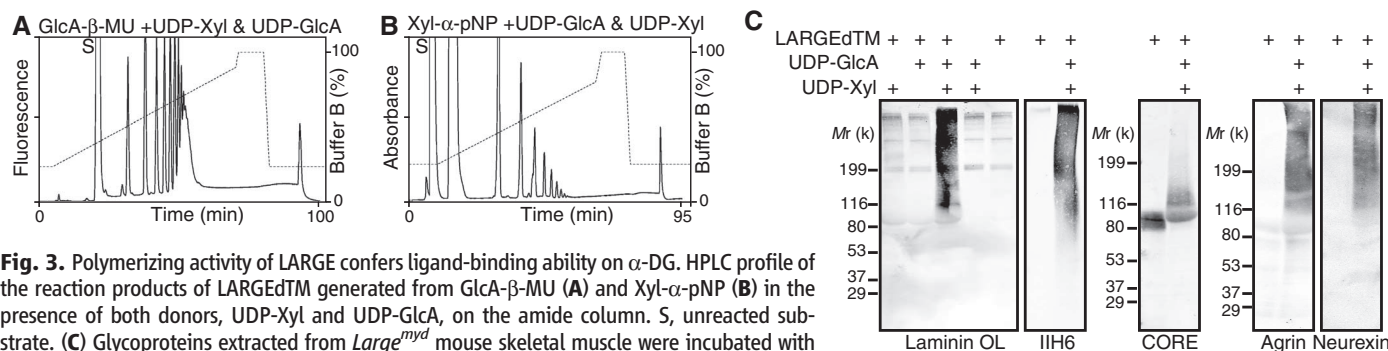


Fig. 3. Polymerizing activity of LARGE confers ligand-binding ability on α -DG. HPLC profile of the reaction products of LARGETM generated from GlcA- β -MU (A) and Xyl- α -pNP (B) in the presence of both donors, UDP-Xyl and UDP-GlcA, on the amide column. S, unreacted substrate. (C) Glycoproteins extracted from *Large*^{myd} mouse skeletal muscle were incubated with LARGETM, with or without UDP-GlcA and UDP-Xyl, and analyzed by immunoblotting with IIH6 or the CORE antibody, or by overlay assays (OLs) using laminin-G domain-containing ECM ligands (laminin, agrin, or neurexin).

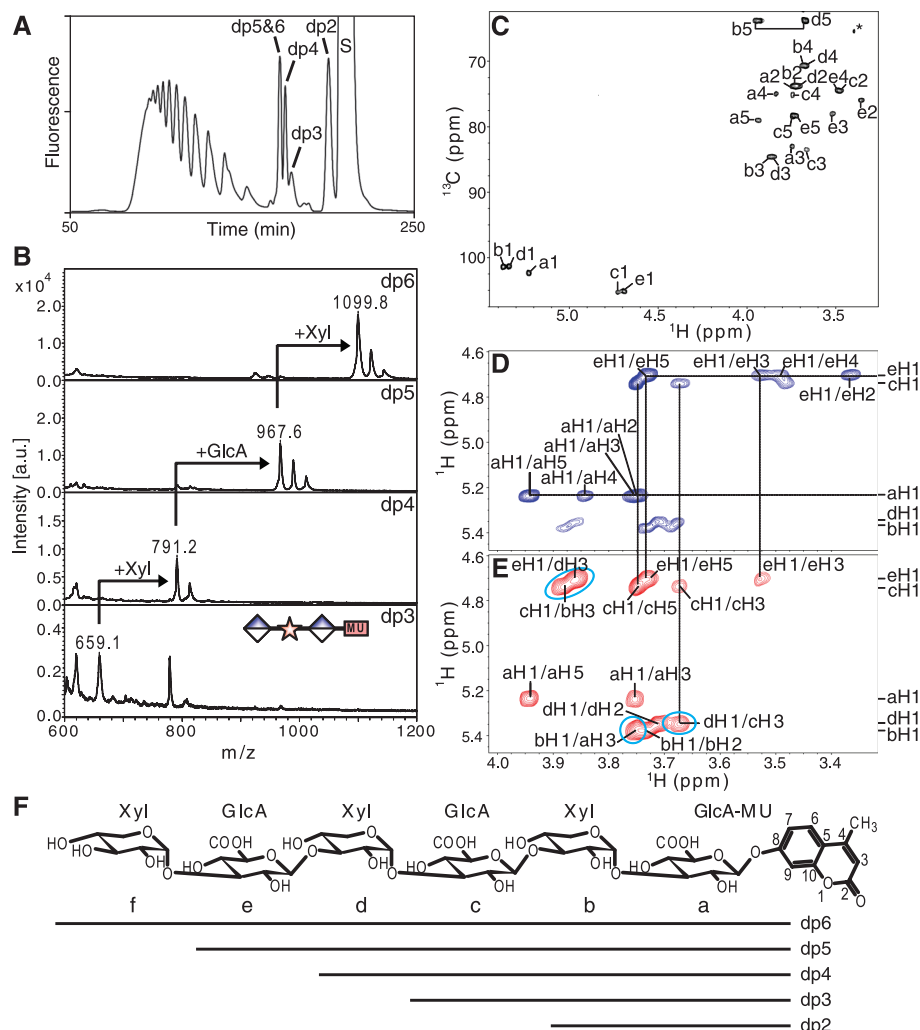


Fig. 4. MS and NMR analyses of products obtained from in vitro LARGE enzymatic reaction products, using GlcA- β -MU as the acceptor and UDP-GlcA and UDP-Xyl as the donors. **(A)** Separation profile of polymeric oligosaccharide by Superdex Peptide 10/300. S, unreacted substrate. **(B)** Matrix-assisted laser desorption-ionization tandem MS analysis of products dp3 to dp6. **(C)** HMQC spectrum of dp5 at 15°C. Assigned cross peaks are labeled with a letter representing the subunit [as designated in (F)], and a number representing the position on that subunit. The cross peak derived from sample impurities is marked by an asterisk. **(D)** TOCSY spectrum of dp5, collected with a mixing time of 120 ms at 15°C. **(E)** ROESY spectrum of dp5, collected with a mixing time of 300 ms at 15°C for the assignment of interglycosidic linkages (indicated by blue circles). The first letter in each label refers to the sugar subunit and the second to the hydrogen position of that subunit. **(F)** Structures of the polymeric oligosaccharides produced by the LARGE enzymatic reaction in vitro, with the sugar subunits labeled a to f.

(15), and anionic sugars distinct from sialic acid are involved (16). Thus, we tested whether LARGE could add Xyl and GlcA to the acceptor on α -DG, by performing the LARGEdTM reaction using both donors. Multiple products were generated for both acceptors (Xyl- α -PNP and GlcA- β -MU) (Fig. 3, A and B). We next asked whether this enzymatic activity could confer ligand-binding ability to α -DG in vitro, using skeletal muscle glycoproteins from the *Large^{myd}* mouse [in which a mutation in the *LARGE* gene causes defects in α -DG glycosylation (17)] as the acceptor substrate. Functional modification was robust only when both UDP-GlcA and UDP-Xyl were present (Fig. 3C). Thus, LARGE can assemble a polysaccharide with

ligand-binding activity onto the immature glycan of the *Large^{myd}* α -DG.

To identify the precise glycan structure that LARGE generates, we performed a large-scale enzymatic reaction with GlcA- β -MU, separated the products by gel filtration (Fig. 4A), and further purified those peaks (fig. S10). MS analysis of the product peaks ranging from a degree of polymerization (dp) of 3 to 6 showed m/z values corresponding to those of the products expected to be sequentially modified by GlcA and Xyl (Fig. 4B). Products dp2 to dp6 were further analyzed by nuclear magnetic resonance (NMR) to determine how the sugars are linked (fig. S11 to S15). Representative $^{13}\text{C}/^1\text{H}$ heteronuclear mul-

tiple quantum coherence spectroscopy (HMQC), total correlation spectroscopy (TOCSY), and rotating-frame Overhauser effect spectroscopy (ROESY) spectra for dp5 are shown in Fig. 4, C to E, respectively. The majority of the spin systems of the sugar residues can be traced and assigned using different TOCSY mixing times together with double-quantum filtered correlated spectroscopy (COSY) spectra (Fig. 4D and fig. S11). GlcA residues c and e were linked via β 1-3 linkages to Xyl residues b and d, respectively (Fig. 4E); and Xyl residues b and d were connected to GlcA residues a and c via α 1-3 linkages, respectively (Fig. 4E). The structures of products dp2 to dp6 are shown in Fig. 4F; complete NMR assignments are listed in table S1. The fact that the glycosidic linkages were preserved among all tested products indicates that LARGE is a polymerizing enzyme with UDP-GlcA: α Xyl β 1,3-GlcA-T and UDP-Xyl: β GlcA α 1,3-Xyl-T activities.

Here, we have identified the enzymatic function of LARGE, which produces a polysaccharide with repeating units of [-3Xyl- α 1,3GlcA β 1-]. We believe that LARGE synthesizes the polymer on the *O*-mannosyl glycan of α -DG in vivo, but can possibly act on other glycans and/or other protein substrates when the enzyme concentration is artificially high. α -DG binds the LG4 and 5 domains of laminin α chains, and it is suggested that three basic patches contribute to these interactions (18). The LARGE-synthesized, negatively charged glycan on α -DG likely binds to laminin through electrostatic associations with these basic patches, a notion supported by the fact that one patch, 2719RKR contributes to binding with heparin, which contains acidic sugars GlcA and iduronic acid. The glycan we have observed resembles heparin-HS- and CS-DS-GAGs, linear polysaccharides that consist of repeating disaccharides and are synthesized by copolymerases with dual glycosyltransferase activities. Our findings may extend the types of GAG that provide a platform to retain molecules onto cell surface and/or in the ECM.

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Supporting Online Material

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Materials and Methods

Figs. S1 to S15

Table S1

References (19–22)

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RNA Elimination Machinery Targeting Meiotic mRNAs Promotes Facultative Heterochromatin Formation

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Facultative heterochromatin that changes during cellular differentiation coordinates regulated gene expression, but its assembly is poorly understood. Here, we describe facultative heterochromatin islands in fission yeast and show that their formation at meiotic genes requires factors that eliminate meiotic messenger RNAs (mRNAs) during vegetative growth. Blocking production of meiotic mRNA or loss of RNA elimination factors, including Mmi1 and Red1 proteins, abolishes heterochromatin islands. RNA elimination machinery is enriched at meiotic loci and interacts with Ctr4/Suv39h, a methyltransferase involved in heterochromatin assembly. Heterochromatin islands disassemble in response to nutritional signals that induce sexual differentiation. This process involves the antisilencing factor Epe1, the loss of which causes dramatic increase in heterochromatic loci. Our analyses uncover unexpected regulatory roles for mRNA-processing factors that assemble dynamic heterochromatin to modulate gene expression.

Heterochromatin assembly is critical for various chromosomal processes (1–3). In fission yeast (*Schizosaccharomyces pombe*), noncoding RNAs (ncRNAs) and RNA interference (RNAi) factors implicated in processing ncRNAs facilitate loading of Ctr4/Suv39h to assemble constitutive heterochromatin domains (4, 5). Ctr4 methylates histone H3 lysine 9 (H3K9me) to create binding sites for chromodomain proteins, including the Chp1 subunit of the Ago1-containing RNA-induced transcriptional gene silencing (RITS) complex, as well as HP1 family proteins Swi6 and Chp2, which associate with chromatin modifiers, including Snf2–histone deacetylase repressor complex (SHREC) involved in transcriptional silencing (3).

Apart from constitutive heterochromatin domains at centromeres, subtelomeres, and mating-type locus, H3K9me and HP1 proteins can also be detected within additional genomic regions at discrete genes in the *S. pombe* genome (6). However, the assembly of heterochromatin targeting genes and its modifications in response to signals that modulate gene expression have not been explored. To address this, we mapped H3K9me across the genome (7).

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Besides previously reported heterochromatin loci, we reproducibly detected H3K9me at several additional sites (Fig. 1A). These heterochromatin islands encompass ~30 loci, which include genes located adjacent to ncRNAs and a few long terminal repeats (Fig. 1B). Overlapping transcription at convergent genes is believed to target H3K9me via RNAi (8), whereas heterochromatin islands correspond to both convergent and nonconvergent loci (Fig. 1, B and C). A distinctive feature of heterochromatin islands is their preferential association with meiotic genes, which are silenced during vegetative growth (table S1). Most H3K9me enrichment corresponds to either open reading frames or 3' ends of genes (Fig. 1B), consistent with transcription-coupled processes targeting H3K9me. Histone H3K4 methylation, a modification linked to RNA polymerase II transcription, could be detected at the 5' ends of genes within heterochromatin islands (fig. S1).

Factors that bind H3K9me and their associated effectors localize to heterochromatin islands: Chromatin immunoprecipitation (ChIP) detected the Ctr4 complex (CtrC) subunit Raf2, Swi6/HP1, as well as posttranscriptional and transcriptional silencing activities, such as RNAi effector complex RITS (Chp1 and Ago1) and SHREC (Clr3 and Mit1), respectively, at heterochromatin islands (fig. S2). Additionally, Dcr1 localizes to three genes corresponding to islands (9). We also found the cohesin-loading factor Mis4, which interacts with Swi6/HP1 (10), and the cohesin sub-

unit Rad21 enriched at meiotic heterochromatin islands (fig. S2).

Our analysis identified the antisilencing factor Epe1 (11, 12) at heterochromatin islands (fig. S2). The loss of Epe1 caused the spread of H3K9me at most heterochromatin islands and at subtelomeric regions (fig. S3, A and B). Moreover, *epe1Δ* cells showed several H3K9me peaks that were not detected in wild-type cells (fig. S3C). More than 30 additional peaks mapped to ~100 convergent and nonconvergent loci. As in the case of the wild type, a major fraction of heterochromatin islands in *epe1Δ* mapped to meiotic genes. Thus, the *S. pombe* genome appears to harbor numerous heterochromatin nucleation sites, but heterochromatin assembly at many of these loci is suppressed by factors such as Epe1.

Given that heterochromatin islands map to transcribed regions, we wondered if RNAi targets heterochromatin to these loci. The loss of Dicer (Dcr1) or Argonaute (Ago1) caused only partial or no reduction in H3K9me at heterochromatin islands except island 5, which showed considerable reduction of H3K9me (fig. S4, A and B). Moreover, de novo targeting of H3K9me to *ssm4* and *mei4* occurred even in the absence of Ago1, albeit at levels lower than those of the wild type (fig. S4C), suggesting that additional RNAi-independent mechanism(s) target heterochromatin to meiotic loci. We also investigated the effects of histone deacetylases (HDACs), including Sir2 and SHREC (3), implicated in heterochromatin formation. Deletion of *sir2* encoding a nicotinamide adenine dinucleotide-dependent HDAC (3) caused defective H3K9me at the majority of islands (fig. S5A), but SHREC subunits were dispensable (fig. S5B).

We next sought to investigate the mechanism of heterochromatin assembly at meiotic loci. Meiotic genes such as *ssm4* are transcribed during vegetative growth, but their transcripts are processed by the exosome (13–16). The loss of Rrp6, an exosome-associated 3'-to-5' exonuclease (17), led to the accumulation of *ssm4* mRNA and a long RNA, which initiated from a promoter upstream of *tpi1* (Fig. 2A and fig. S6). When production of *ssm4* mRNA and the long RNA was disrupted by insertion of *ura4* transcription terminator at the *ssm4* promoter (Fig. 2A), the resulting strain showed the loss of H3K9me at the *ssm4* (Fig. 2B). We also generated a strain in which *ura4* terminator specifically blocks long RNA, without affecting *ssm4* mRNA (fig. S7). *ssm4* mRNA was sufficient to nucleate heterochromatin, as disruption of the long RNA did not abolish H3K9me (fig. S7). Based on these data,

transcription is required for H3K9me at the *ssm4* meiotic gene.

The elimination of meiotic mRNAs in vegetative cells involves the YTH domain-containing protein Mmi1 (13). Mmi1 binds mRNAs containing determinant of selective removal (DSR) sequences and mediates their degradation by the exosome (13, 16). Meiotic mRNA suppression also requires the Red1 protein, which interacts with Mmi1, the exosome, and pre-mRNA 3'-processing factors (15). We wondered if the RNA elimination machinery targets H3K9me. Insertion of the *mei4* DSR at the 3' untranslated region

of *ura4* resulted in H3K9me at this site, especially when *ura4*-DSR was expressed (Fig. 2C), and *ssm4* lacking its DSR failed to nucleate H3K9me (fig. S8). More importantly, the loss of Mmi1, Red1, or Rrp6, which caused accumulation of DSR-containing transcripts (fig. S9A) (13, 15), abolished H3K9me at both *ssm4* and *mei4*, as determined by ChIP and ChIP-chip (Fig. 2, D and E). At other meiotic loci, we also observed defects in H3K9me in these mutants (Fig. 3A and fig. S10A), but we saw no change at heterochromatin islands targeting nonmeiotic loci, such as islands 14 and 15 (Fig. 3B). Based

on these results, RNA elimination factors are required for the assembly of heterochromatin at meiotic genes.

Consistent with RNA elimination factors directly promoting H3K9me, we found Red1 and Rrp6 enriched at *ssm4* and *mei4* (Fig. 2F and fig. S9B). Red1 was also detected at heterochromatin islands at other meiotic genes (Fig. 3A). Localization of Red1 correlated with its requirement for H3K9me at individual loci (Fig. 3A and fig. S10A). Red1 could not be detected at islands that showed no reduction in H3K9me in *red1Δ* cells (Fig. 3B and fig. S10A). *rec8* and *spo5* genes,

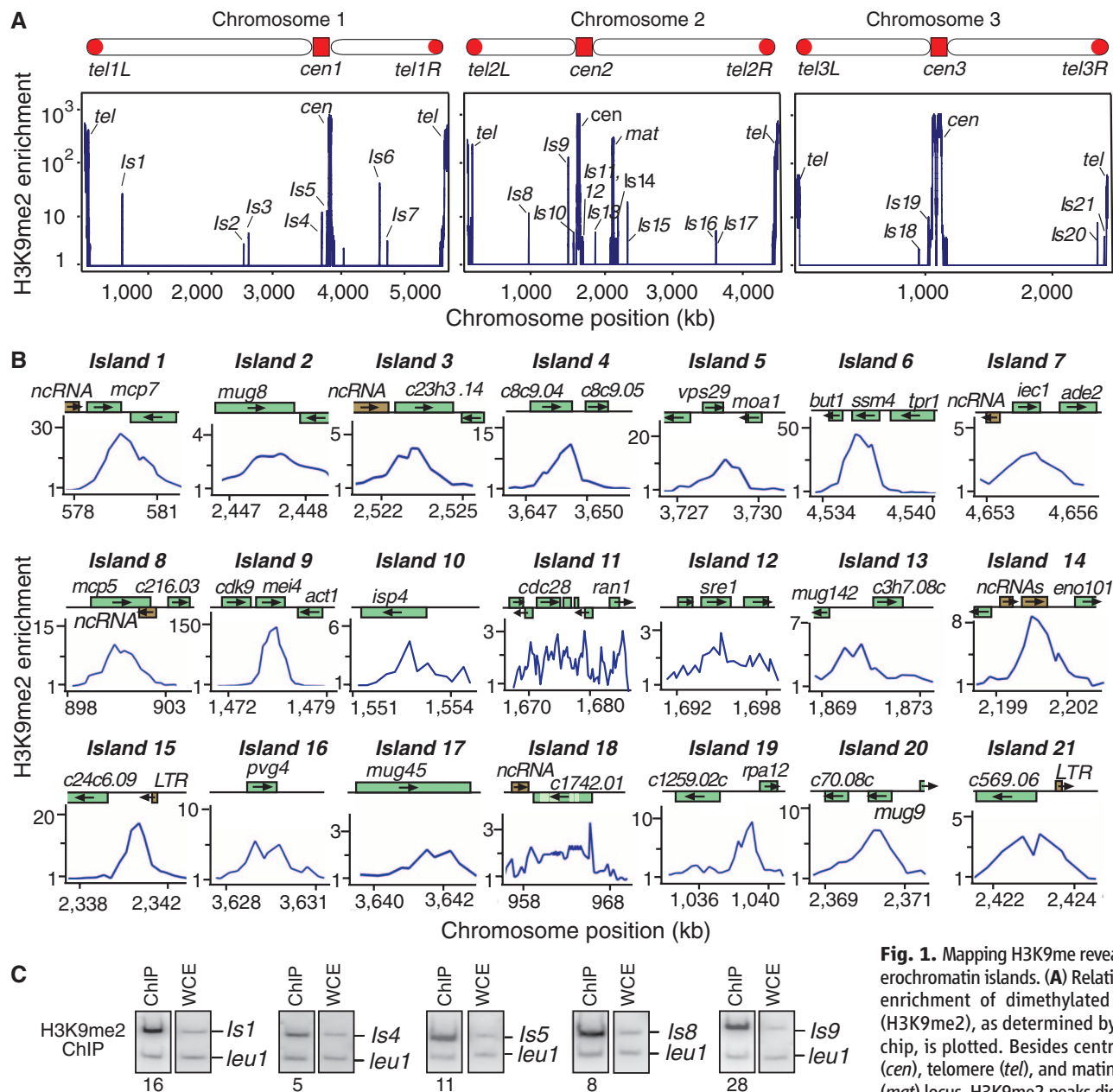


Fig. 1. Mapping H3K9me reveals heterochromatin islands. (A) Relative fold enrichment of dimethylated H3K9 (H3K9me2), as determined by ChIP-chip, is plotted. Besides centromere (*cen*), telomere (*tel*), and mating-type (*mat*) locus, H3K9me2 peaks distribute across the genome (islands 1 to 21).

(B) H3K9me2 distribution at individual loci. Chromosome positions in (A) and (B) correspond to Sanger Center Pombe database 2004 assembly. LTR, long terminal repeat. (C) ChIP confirms H3K9me2 enrichment at selected loci. DNA isolated from immunoprecipitated chromatin (ChIP) and whole-cell crude extract (WCE) was used to perform multiplex polymerase chain reaction. Relative fold enrichment values are shown.

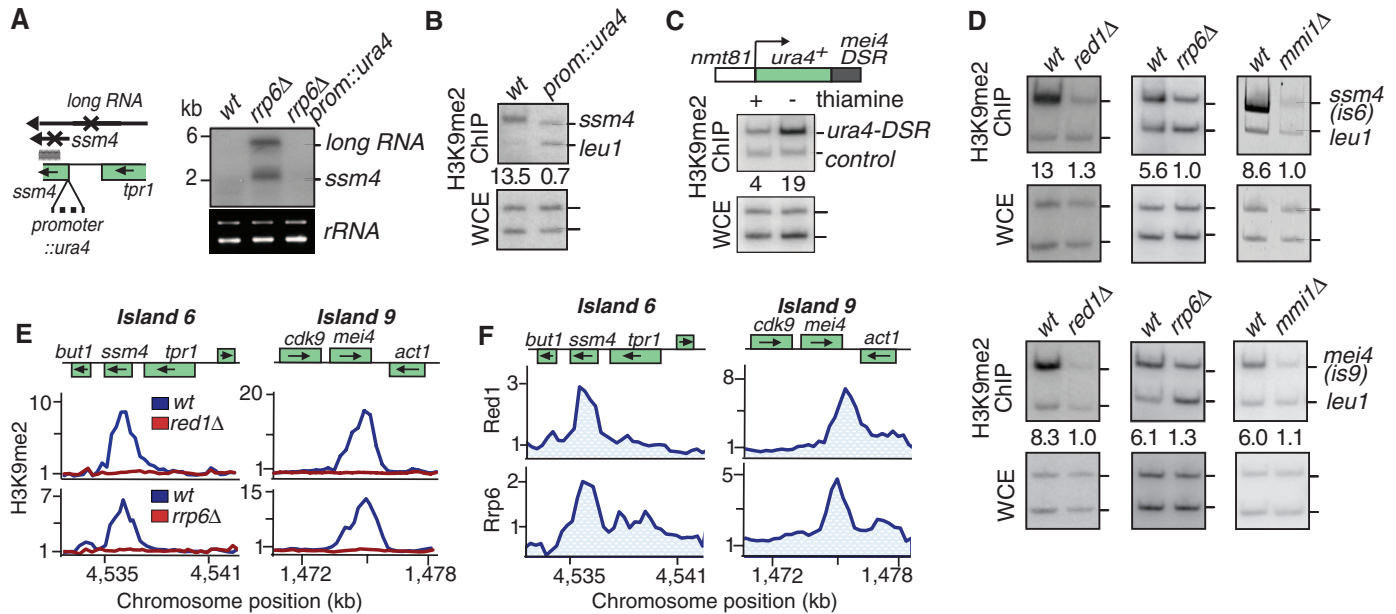


Fig. 2. RNA elimination machinery affects heterochromatin assembly at meiotic genes. (A) Insertion of a mini-*ura4* at the *ssm4* promoter (*prom::ura4*) blocks the production of *ssm4* mRNA and a long RNA, as determined by Northern blot analysis. *rrp6Δ* is used to facilitate RNA detection. Ribosomal RNA (*rRNA*) serves as a loading control. *wt*, wild type. (B) Blocking *ssm4* transcription abolishes H3K9me at this locus. H3K9me2 levels were assayed by ChIP. (C) *mei4* DSR

induces H3K9me at an ectopic site. DSR fused to *ura4⁺* was expressed under the control of thiamine-repressible *nmt1* promoter. Cells grown in the presence or absence of thiamine were used to detect H3K9me by ChIP. Endogenous *ura4* serves as a control. (D and E) *mmi1Δ*, *red1Δ*, or *rrp6Δ* affect H3K9me at *ssm4* and *mei4*. Results of ChIP (D) or ChIP-chip (E) are presented. (F) Red1-myc and Rrp6-myc distribution at *ssm4* and *mei4*, as determined by ChIP-chip, are plotted.

which encode DSR-containing transcripts degraded by a Mmi1-based mechanism (13, 16), lack detectable levels of H3K9me (fig. S10B). The lack of H3K9me enrichment correlated with the absence of Red1 binding at these loci (fig. S10B). The correlation between localization of RNA elimination factors and H3K9me prompted us to investigate interactions between these factors. Immunoprecipitation analyses detected RNA elimination factor Red1 interacting with Clr4 and another ClrC subunit, Raf1, and that these interactions were not sensitive to deoxyribonuclease I or ribonuclease A treatment (Fig. 3C). These data demonstrate that Red1 is a component of a protein network involved in the elimination of meiotic RNAs that recruits Clr4 to assemble heterochromatin islands.

Heterochromatin and its associated factors such as RNAi components might promote meiotic gene silencing. Loss of Ago1 alone did not cause detectable changes in *ssm4* and *mei4* silencing (fig. S11). However, the effects of RNAi machinery might be masked by the activity of the exosome. Because the exosome is required for both mRNA processing and H3K9me, it was not possible to study the effects of heterochromatin factors in the *rrp6Δ* background. However, the degradation of meiotic mRNAs by the exosome requires the poly(A) binding protein Pab2 (16, 18), which does not interact with Red1 (15). *pab2Δ* impaired the degradation of transcripts but maintained considerable levels of H3K9me at meiotic loci (Fig. 3, D and E). When *pab2Δ* was combined with *clr4Δ*, the resultant double mutant

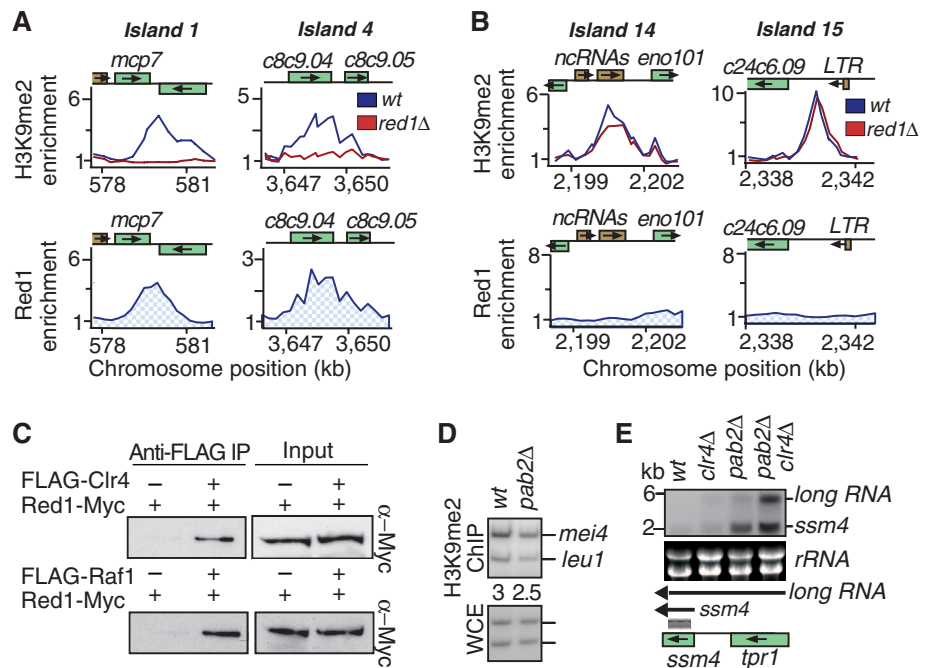
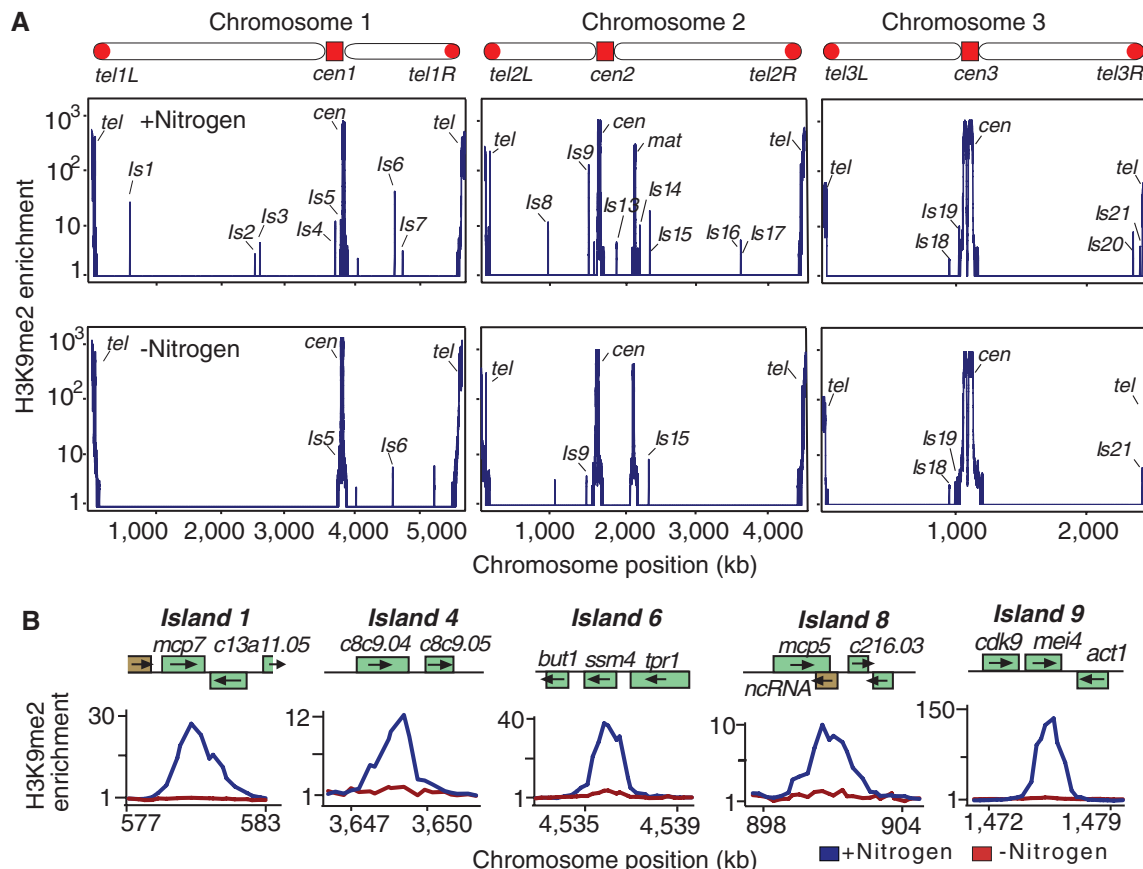


Fig. 3. H3K9me at meiotic loci requires Clr4-interacting Red1 protein. (A and B) Relative enrichments of H3K9me2 and Red1-myc are plotted. (A) Red1 localized to islands 1 and 4 and is required for H3K9me2 at these loci. (B) *red1Δ* has no effect on H3K9me2 at islands 14 and 15, which lack detectable levels of Red1. (C) Clr4 and Raf1 interact with RNA processing factor Red1. Immunoprecipitation of FLAG-Clr4 or FLAG-Raf1 was followed by Western blotting with myc antibody to detect Red1-myc. IP, immunoprecipitation. (D) *pab2Δ* causes only minor change in H3K9me at *mei4*, as determined by ChIP. (E) Clr4 and Pab2 act in parallel pathways to silence meiotic genes and to suppress long RNA. RNA from indicated strains was used to perform Northern blotting with an *ssm4* probe (black bar).

Fig. 4. Nutritional signals that induce meiosis trigger the loss of heterochromatin islands. **(A and B)** Nitrogen starvation causes reduction in H3K9me2 at meiotic heterochromatin islands. Levels of H3K9me2 measured by ChIP-chip performed using cells cultured in the presence or absence of nitrogen are plotted using a logarithmic (A) and linear (B) scale.



showed cumulative accumulation of *ssm4* mRNA and long RNA (Fig. 3E). These results suggest a role for Clr4 in silencing meiotic genes during vegetative growth. Clr4 might directly promote RNA processing (19) and/or facilitate loading of silencing effectors, such as chromatin modifiers and the RNAi machinery (fig. S2), via H3K9me-bound chromodomain proteins. Indeed, *clr4Δ* cells are defective in the localization of Swi6 and the RITS subunit Chp1 at *ssm4* and *mei4* loci (6).

Meiotic gene expression is induced in response to sexual differentiation signals (20). Nitrogen starvation arrests cells in G₁ phase of the cell cycle and triggers sexual differentiation. However, growth arrest can be reversed by the resupply of nitrogen. Nitrogen deprivation caused severe reduction in H3K9me at meiotic genes but had no effect on heterochromatin at centromeres, telomeres, and the *mat* locus (Fig. 4, A and B, and fig. S12A). The observed effect was specific to nitrogen starvation and was not observed when cells were arrested in G₁ phase (fig. S12B). Appreciable reduction in H3K9me could be detected after 4 hours of nitrogen starvation (fig. S13A). The rapid loss of H3K9me involves Epe1, because disassembly of heterochromatin islands is delayed in *epe1Δ* mutant (fig. S13A). Resupplying nitrogen to starved cells restored heterochromatin to meiotic loci within 24 hours after the addition of nitrogen source to growth me-

dium (fig. S13B). Thus, heterochromatin islands at meiotic loci are developmentally regulated.

Our analysis expands the repertoire of loci targeted by heterochromatin and uncovers a mechanism for heterochromatin assembly. Heterochromatin formation at meiotic loci requires transcription but can occur independent of RNAi or a specific gene orientation. We suggest that Mmi1 binding to DSR-containing RNAs engages Red1 and the exosome to nucleate heterochromatin by targeting Clr4/Suv39h (fig. S14). Because Red1-exosome degrades many RNAs and certain Mmi1 targets (e.g., *rec8* and *spo5*) lack H3K9me, it is likely that additional factors, including specific features of RNAs, are important for loading heterochromatin. In this regard, Red1 and Mmi1-cooperate with pre-mRNA 3'-processing factors to promote hyperadenylation of target transcripts, which is critical for meiotic gene silencing (15, 16). Red1 and 3'-processing factors may be components of a mechanism that couples degradation of meiotic RNAs to heterochromatin assembly. Note that the Rik1 subunit of the Clr4 complex, which suppresses accumulation of aberrant RNAs (19), resembles cleavage and polyadenylation specificity factors involved in pre-mRNA 3'-processing (21). Similar to facultative heterochromatin in higher eukaryotes, heterochromatin islands are remodeled in response to cellular differentiation signals. These results suggest that signaling mechanisms exist that feed into heterochromatin pathways

to facilitate coordinated changes in gene expression and reprogram the genome for other chromosomal events during meiotic induction. Similar pathways involving cotranscriptional RNA processing factors might promote RNA- and transcription-coupled epigenetic modification observed in other systems (22–25).

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Asymmetry and Aging of Mycobacterial Cells Lead to Variable Growth and Antibiotic Susceptibility

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Cells use both deterministic and stochastic mechanisms to generate cell-to-cell heterogeneity, which enables the population to better withstand environmental stress. Here we show that, within a clonal population of mycobacteria, there is deterministic heterogeneity in elongation rate that arises because mycobacteria grow in an unusual, unipolar fashion. Division of the asymmetrically growing mother cell gives rise to daughter cells that differ in elongation rate and size. Because the mycobacterial cell division cycle is governed by time, not cell size, rapidly elongating cells do not divide more frequently than slowly elongating cells. The physiologically distinct subpopulations of cells that arise through asymmetric growth and division are differentially susceptible to clinically important classes of antibiotics.

Tuberculosis, caused by infection with *Mycobacterium tuberculosis*, remains a major global health problem, killing more than 1.5 million people annually. Although antibiotic therapy rapidly reduces the bacterial burden, eliminating the infection requires long courses

of multiple antibiotics (1, 2). It has been suggested that this lengthy course of treatment is required because the mycobacterial population is functionally heterogeneous and contains cells that are differentially susceptible to antibiotics, nonreplicating, or sequestered in the body (3, 4).

Because almost all antibiotics target bacterial processes involved in cell growth, we hypothesized that there is heterogeneity in growth states of mycobacterial cells that underlies differential antibiotic susceptibility.

To measure the growth and antibiotic susceptibility of mycobacteria at a single-cell level, we designed a microfluidic chamber to culture mycobacteria for live-cell imaging. This device allows cell movement in two dimensions but constrains bacteria to a single focal plane (Fig. 1A). Cells are grown in shallow channels that are connected to a perpendicular media channel, which provides a homogeneous mixture of nutrients by diffusion. This device enables imaging of mycobacterial growth for up to five generations, at which point images become too crowded to score (Fig. 1B). In this device, we can expose

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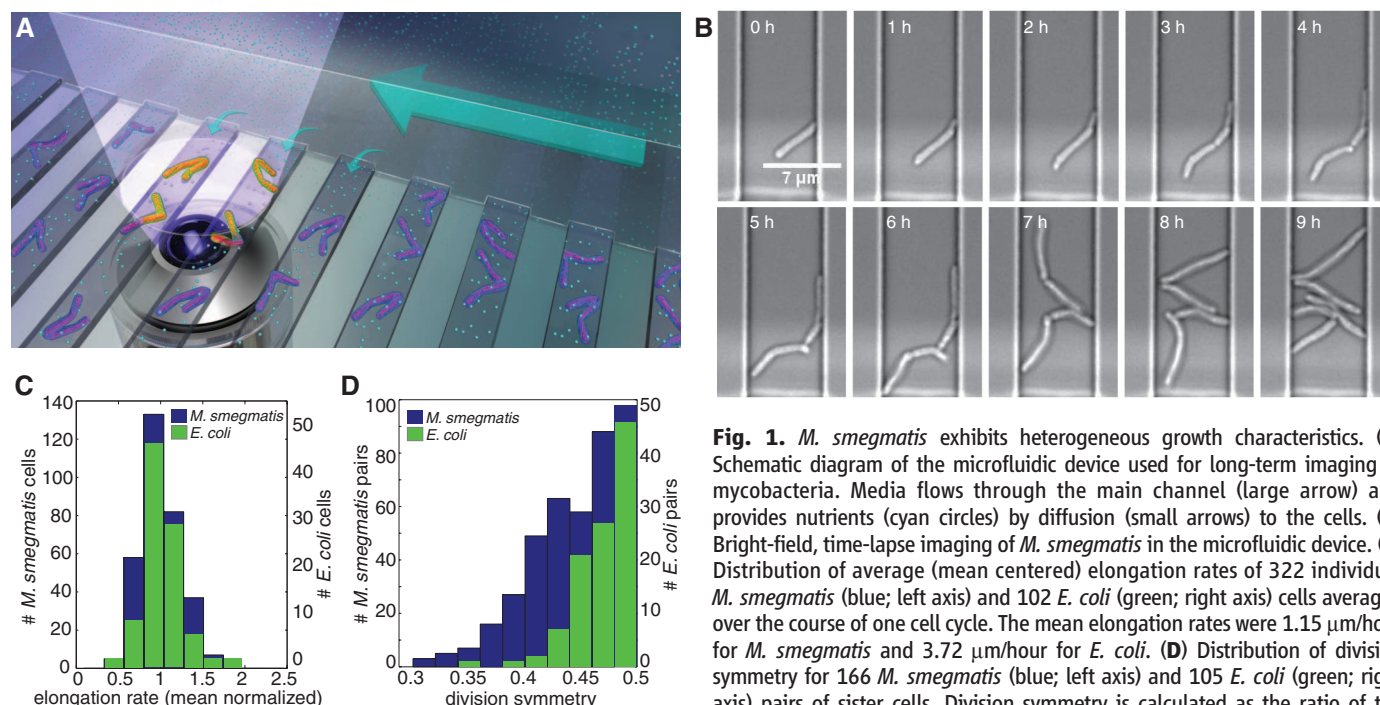


Fig. 1. *M. smegmatis* exhibits heterogeneous growth characteristics. (A) Schematic diagram of the microfluidic device used for long-term imaging of mycobacteria. Media flows through the main channel (large arrow) and provides nutrients (cyan circles) by diffusion (small arrows) to the cells. (B) Bright-field, time-lapse imaging of *M. smegmatis* in the microfluidic device. (C) Distribution of average (mean centered) elongation rates of 322 individual *M. smegmatis* (blue; left axis) and 102 *E. coli* (green; right axis) cells averaged over the course of one cell cycle. The mean elongation rates were 1.15 $\mu\text{m}/\text{hour}$ for *M. smegmatis* and 3.72 $\mu\text{m}/\text{hour}$ for *E. coli*. (D) Distribution of division symmetry for 166 *M. smegmatis* (blue; left axis) and 105 *E. coli* (green; right axis) pairs of sister cells. Division symmetry is calculated as the ratio of the length of the smaller sister to the sum of the lengths of both sisters at division.

cells to defined stressors such as antibiotics and follow the responses of individual cells. As the growth and division machinery is highly conserved between pathogenic and nonpathogenic mycobacteria, we focused our live-cell imaging

experiments on the experimentally tractable, model mycobacterium, *Mycobacterium smegmatis*.

To assess population variation in growth states, we measured the elongation rates of individual *M. smegmatis* cells grown in rich medium. As a

point of comparison, we also measured the growth parameters of individual *Escherichia coli* cells grown in rich medium in our microfluidic device. We found significantly more variability in the elongation rates of mycobacterial cells compared to *E. coli* cells (Fig. 1C; $F < 0.05$) (5). Mycobacteria lack the molecular rulers that ensure symmetric cell division, which place the division septum in the center of the cell in other rod-shaped organisms such as *E. coli* and *Bacillus subtilis* (6). Thus, we wondered whether the variability in mycobacterial elongation rates was related to asymmetry in cell division (7, 8). We therefore assessed the symmetry of mycobacterial cell division and found that cell division is significantly less symmetric in *M. smegmatis* than in *E. coli* (Fig. 1D; $F < 0.001$) (5). We observed similar asymmetry in cell division in *M. tuberculosis* (fig. S1).

Asymmetry in cell elongation could cause apparent asymmetry in cell division and subsequent variability in the elongation rates of daughter cells. To assess this possibility, we took advantage of the fact that mycobacteria elongate at their poles rather than along the lateral cell body as in *E. coli* (6, 9). This allowed us to quantify cell elongation by pulse labeling the cell wall with a fluorescent amine-reactive dye and measuring the extension of the unlabeled poles (Fig. 2A) (10). Notably, we found that mycobacterial cells elongate preferentially at the old pole (Fig. 2, B and C). In static images, unipolar growth produces a “cigar-band” of cell wall labeling with the amine-reactive dye where one pole has elongated significantly more than the other, which we also observe in *M. tuberculosis* (Fig. 2D).

Unipolar growth per se does not explain cell-to-cell variability in elongation rates or cell sizes, but it does create different types of cells at division. One daughter cell inherits the growing pole, whereas the other daughter cell must create a new growth pole after every division (schematic in Fig. 2E). The new growth pole is generated at the older pole (opposite the division septum), and therefore the direction of growth changes with every cell cycle. We have quantified this for a single, representative cell over four generations in Fig. 2E. By contrast, in the daughter cell that inherits the growing pole (indicated by an arrow in Fig. 2E), elongation continues from the inherited growth pole (fig. S2).

We hypothesized that the daughter cell inheriting the growth pole would elongate at a different rate than its sister cell, which must assemble a new growth pole. We tested this hypothesis by computing the differences in elongation rate between pairs of sister cells. We found that on average, the sister cell inheriting the growth pole elongates faster than the sister cell that establishes a new growth pole (Fig. 3A; $P < 0.05$). The cell inheriting the growth pole is also longer at birth than its sister cell, consistent with a model in which elongation remains asymmetric during septation (Fig. 3B; $P < 0.05$). Thus, each division results in two distinctive sister cells. We term

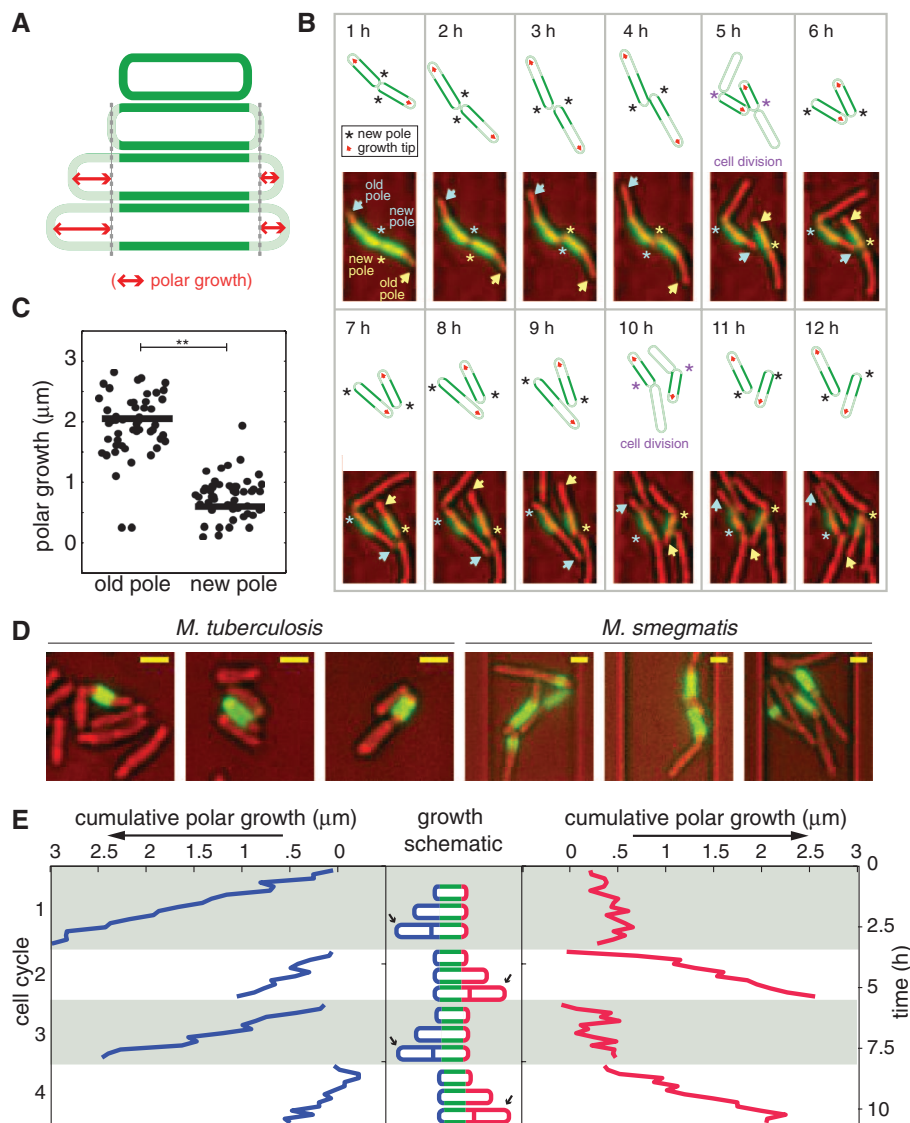


Fig. 2. *M. smegmatis* growth is asymmetric and elongation occurs from the old pole. (A) Schematic diagram of the pulse-chase experiment used to measure polar growth. Cells were labeled with amine-reactive dye (green), and growth was assessed by measuring the extension of the unlabeled region (red arrows). Cell-wall labeling did not alter cell elongation rate (fig. S5A), other labeling chemistries such as hydroxylamine labeling via periodate oxidation led to similar staining patterns (data not shown), and similar labeling patterns were seen in cells grown in broth and in microfluidic channels (fig. S5B). (B) Time-lapse imaging of two sister cells after the pulse labeling (green) of the cell wall. The bright-field images were pseudo-colored red. New poles are annotated with asterisks and the old poles with arrows. Each cell's poles are annotated with the same color asterisk and arrow. A schematic is drawn above each image, marking the new poles and growth poles with an asterisk and red arrow, respectively. We also used a fluorescein-vancomycin conjugate (Van-FL) to stain nascent peptidoglycan (30) and found preferential labeling of the old pole over the new pole (fig. S5C). (C) Growth over one cell cycle at new versus old poles in 50 cells ($*P < 0.001$ by a Mann-Whitney rank sum test). There was no cell in which the new pole elongated more than the old pole. (D) Three representative images of *M. tuberculosis* (left) and *M. smegmatis* (right) after the pulse-labeling (green) of the cell wall (after 48 hours in broth culture for *M. tuberculosis* and 6 hours in the microfluidic device for *M. smegmatis*). The bright-field images are pseudo-colored red. Scale bars represent 1.3 μm. Comparison images of *E. coli* labeled under similar conditions are in fig. S5D. (E) Cumulative polar growth for one labeled cell plotted for each pole through four cell cycles. The cell inheriting the new pole alternates the direction of growth after division events.

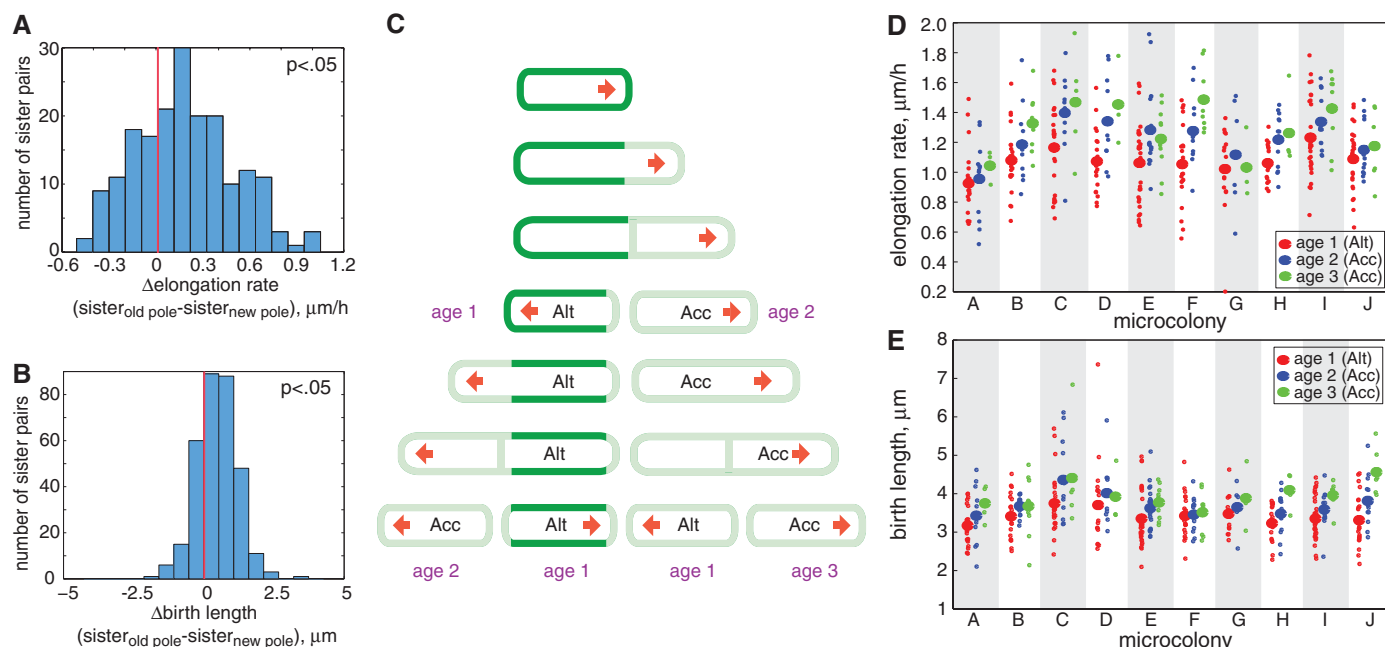


Fig. 3. Division creates sister cells with different growth properties. **(A and B)** Distribution of the differences in elongation rate (A) and birth length (B) between sister cells inheriting the old pole and the new pole. The distributions are skewed ($P < 0.05$; red lines denote zero), indicating that the sister inheriting the older pole elongates faster (in 71% of the 161 sister pairs) and is larger at division (in 74% of the 161 sister pairs). In 7.5% of the 161 sister pairs, the sister that inherited the new pole elongated faster and was longer at birth than its sister. **(C)** Schematic model for mycobacterial growth. A labeled cell (green) is shown to elongate from one pole (red arrow). Two sister cells are created at division: an accelerator (Acc) cell inheriting the old (growing) pole

and an alternator (Alt) cell inheriting the new pole. Growth pole age (in generations) is labeled in purple. **(D and E)** Elongation rate (D) and birth lengths (E) are plotted for 10 microcolonies, with cell subpopulations grouped by growth pole age. Growth pole age was scored by mapping the pedigrees of unlabeled cells through several generations via live-cell imaging. Subpopulation means (large ovals) are plotted along with data from individual cells (small circles). Elongation rate and birth length increase within a colony as the growth poles age ($P < 0.05$ for alternator versus accelerator elongation rates; and $P < 0.05$ for alternator versus accelerator and age 2 versus age 3 birth lengths).

these cells “accelerators,” which inherit the mother’s growth pole and tend to elongate faster, and “alternators,” which must regenerate a new growth pole and tend to elongate more slowly (Fig. 3C).

By definition, all alternator cells have new growth poles, whereas accelerator cells inherit growth poles of varying ages. Some accelerator cells inherit growth poles created in the previous generation, and others inherit growth poles created several generations earlier. To understand whether growth pole age affects the elongation rate of accelerator cells, we mapped the pedigrees of single cells. We assigned an “age” to a cell based on the number of generations its growth pole had experienced; alternator cells have an age of 1 and accelerator cells have an age of 2 or greater (Fig. 3C). We then compared the elongation rate of cells of different ages in populations arising from a single cell, which we term a “microcolony.” Cells with older growth poles elongate faster than cells with younger growth poles (Fig. 3D, $P < 0.05$ for accelerator versus alternator cells). In addition, the birth length of cells increases as the growth pole matures (Fig. 3E, $P < 0.05$ for accelerator versus alternator cells and age 3 versus age 2 cells). Taken together, these data suggest that as the growth pole matures, cells elongate faster and are larger. We occasionally observed cells with older growth poles that elongated more slowly than cells with youn-

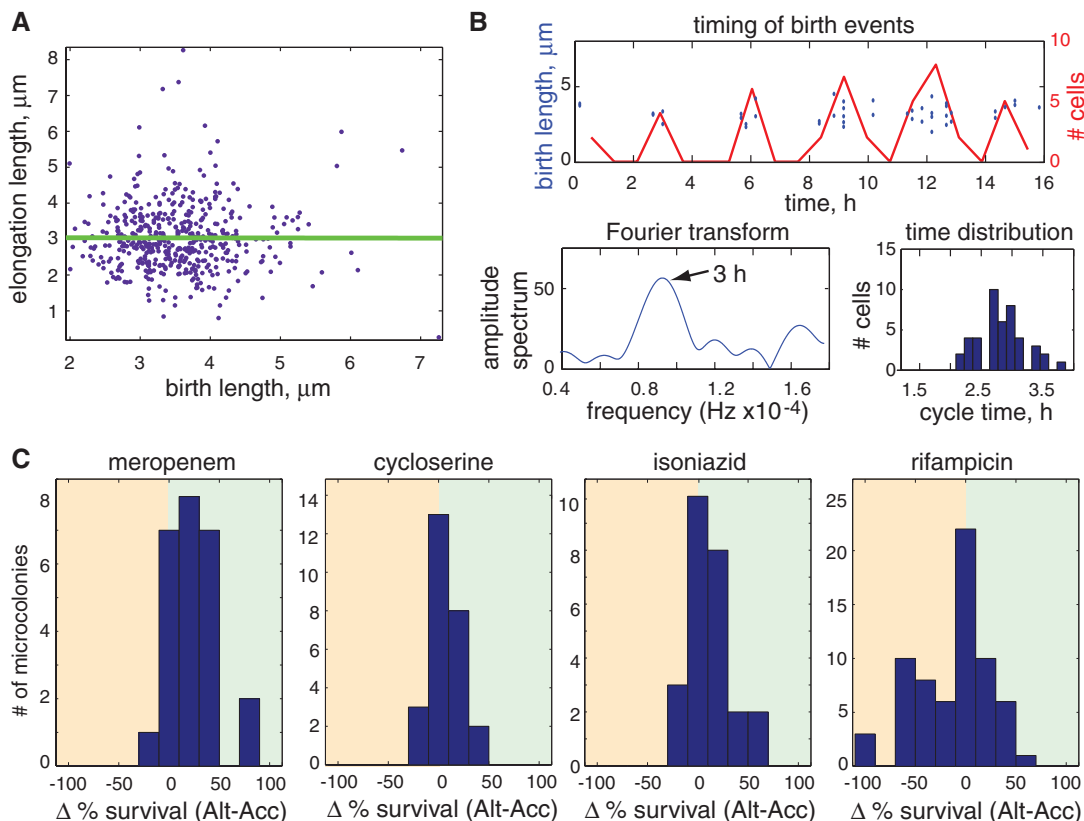
ger growth poles in the same microcolony (e.g., Fig. 3D, colony G), suggesting there may be a mechanism to “reset” the elongation rate.

These data show that the mycobacterial growth pattern generates a population of cells that is heterogeneous in size and elongation rates. We assessed whether rapidly elongating cells also divide more frequently than slowly elongating cells, as would be expected if mycobacteria control cell cycle entry using size-based regulation like *E. coli* (11, 12). Alternatively, mycobacteria might regulate entry into the cell cycle using a time-based mechanism. In yeast, investigators have differentiated between size- and time-based cell cycle regulation using the relationship between birth length and elongation length (13, 14). In cells that use size-based cell cycle regulation, small cells must grow more before dividing, causing the birth length to be negatively correlated with elongation length, whereas in time-based regulation, these lengths are uncorrelated. We therefore measured the association between the birth and elongation lengths of *M. smegmatis* cells. We found no correlation between the two lengths in *M. smegmatis* (regression line slope of 0.00; Fig. 4A), whereas in *E. coli* we found these lengths to be negatively correlated, as expected (regression line slope of -0.75 ; fig. S3A). These data suggest that the mycobacterial division cycle, which we use as a measure of the cell cycle more broadly,

is regulated by time rather than size. Consistent with time-based regulation of cell cycle progression, cell division is synchronized in a microcolony, with closely related cells dividing at similar times (Fig. 4B and fig. S3B). We calculated the microcolony division cycle length by characterizing the distribution of division events in time and in the frequency domain using a Fourier transform (Fig. 4B and fig. S3B). The major frequency corresponds to the division cycle length (and is unbiased by the increasing number of cells as the colony grows), and the amplitude of the peak is a metric for synchronization in the colony. The colony cycle times calculated from the mean and the frequency domain are very similar (within 0.2 hours for each microcolony). However, there were significant differences in cycle times between some microcolonies (fig. S3C). These data suggest that as yet unidentified factors may modulate the cell cycle timer, compounding the diversity of elongation states within the population. Furthermore, the cell cycle length is much longer in slow-growing mycobacteria (~ 22 hours in *M. tuberculosis* as compared to ~ 3 hours in *M. smegmatis*). It is also therefore possible that growth and division cycle timing in *M. tuberculosis* is subject to additional layers of regulation that are not reflected in these studies.

Thus, asymmetric elongation and cell division and a timed cell cycle quickly create a population

Fig. 4. Population heterogeneity of growth characteristics is maintained by time-based cell division cycle regulation and contributes to differential susceptibility to antibiotic stress. **(A)** Birth length and elongation length, which is defined as the length that the cell elongates between birth and division, are uncorrelated for 322 *M. smegmatis* cells (regression slope of 0.00), suggesting that mycobacteria use time to regulate their cell cycle. **(B)** Cell cycle timing is characterized for one microcolony (a second representative microcolony is shown in fig. S3B). Division events are plotted in the time domain as discrete events (blue circles; left axis) and as a histogram (red line; right axis). The histogram of birth events was used to generate an amplitude spectrum with a Fourier transform (lower left). The peak represents the cycle time of the microcolony (arrow). The spread of cycle times for individual cells is shown as a histogram (lower right).



percentage of cells that regrew after antibiotic was removed. The analysis includes 317 cells in 25 microcolonies for meropenem, 374 cells in 26 microcolonies for cycloserine, 334 cells in 25 microcolonies for isoniazid, and 453 cells in 66 microcolonies for rifampicin.

of mycobacterial cells that vary in their elongation rates, sizes, and perhaps other physiologic properties. Because antibiotics target processes essential for growth and division, we hypothesized that these cells might be differentially sensitive to antibiotics. We therefore sought to determine the susceptibility of alternator and accelerator cells to treatment with different classes of antibiotics. To do this, we used live-cell imaging to establish the pedigrees of growing cells and challenged them with the indicated antibiotics at the minimum inhibitory concentrations. We identified bacterial survival by scoring for posttreatment regrowth in 25 to 66 independent microcolonies. Because we observed variability between microcolonies in the efficacy of some antibiotics, especially isoniazid and rifampicin, we calculated the difference in bacterial survival between accelerator and alternator cells for each microcolony and assessed the distribution of this differential across all microcolonies.

Given their different rates of elongation and potential differences in cell wall composition, we predicted that accelerator cells would be more susceptible to cell wall synthesis inhibitors than alternator cells. Indeed, accelerator cells were significantly more sensitive than alternator cells to the peptidoglycan synthesis inhibitors cycloserine and meropenem (Fig. 4C and fig. S4; $P < 0.05$). Accelerator cells were also more sensitive

than alternator cells to isoniazid, which blocks synthesis of cell wall mycolic acids, although there was more variability between microcolonies in the effectiveness of isoniazid (Fig. 4C and fig. S4; $P < 0.05$). However, accelerator cells are not universally more susceptible to antibiotic treatment. Notably, when microcolonies were treated with rifampicin, which inhibits RNA polymerase, alternator cells were more susceptible than accelerator cells (Fig. 4C and fig. S4; $P < 0.05$). As in the case of isoniazid, there was variability among microcolonies in response to rifampicin, suggesting that other potentially heritable factors may also contribute to a cell's susceptibility to these drugs. Thus, we find that alternator and accelerator cells vary in their susceptibility to different classes of antibiotics, consistent with the model that asymmetric growth and division creates physiologically distinct subpopulations of cells.

We have shown here that growth and division in mycobacteria are distinct from that of better-characterized model bacteria. Although unusual, the mycobacterial growth pattern contains spatial and temporal elements that are similar to those used by other bacteria to achieve rapid functional diversification of closely related cells (15–27). The most obvious example of this is *Caulobacter crescentis*, which also exploits polar asymmetry, asymmetry at cell division, and age-dependent

changes in pole function to rapidly create a dimorphic population (15, 22–25). In these organisms and in mycobacteria, variable growth and division patterns create deterministic population diversity at a very high frequency. Mycobacteria may also use other lower-frequency mechanisms, both deterministic and stochastic, to generate a tapestry of cell types (26–29). We anticipate that if these mechanisms for bacterial diversification operate in *M. tuberculosis* as they do in *M. smegmatis*, they may contribute to the highly variable outcomes of tuberculosis infection and treatment.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1216166/DC1
Materials and Methods
Figs. S1 to S5
References (31–36)

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Langerhans Cells Facilitate Epithelial DNA Damage and Squamous Cell Carcinoma

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Polyaromatic hydrocarbons (PAHs) are prevalent, potent carcinogens, and 7,12-dimethylbenz[*a*]anthracene (DMBA) is a model PAH widely used to study tumorigenesis. Mice lacking Langerhans cells (LCs), a signatory epidermal dendritic cell (DC), are protected from cutaneous chemical carcinogenesis, independent of T cell immunity. Investigation of the underlying mechanism revealed that LC-deficient skin was relatively resistant to DMBA-induced DNA damage. LCs efficiently metabolized DMBA to DMBA-*trans*-3,4-diol, an intermediate proximal to oncogenic *Hras* mutation, and DMBA-treated LC-deficient skin contained significantly fewer *Hras* mutations. Moreover, DMBA-*trans*-3,4-diol application bypassed tumor resistance in LC-deficient mice. Additionally, the genotoxic impact of DMBA on human keratinocytes was significantly increased by prior incubation with human-derived LC. Thus, tissue-associated DC can enhance chemical carcinogenesis via PAH metabolism, highlighting the complex relation between immune cells and carcinogenesis.

Epithelial tissues, including skin, are situated at critical junctures with the environment and repeatedly exposed to chemical

toxins and mutagens. In humans, ~90% of cancers arise in epithelial tissues. Such tissues are commonly replete with associated dendritic cells (DCs), for which the prototype is the epidermal Langerhans cell (LC) network. DCs have long been viewed as the primary means by which peripheral tissue neo-antigens are internalized, processed, and presented to antigen-specific T cells that may then mount and coordinate immunoprotection. Consistent with this, LCs induce contact hypersensitivity (CHS) responses and are considered, along with other tissue-resident DCs, to be well-placed to limit carcinogenesis through presentation of tumor-associated antigens to T cells. Recent studies of several LC-mutant mouse strains, however, have collectively argued for a reevaluation of the major functional contributions of LCs to epidermal biology (1–3). For example, CHS responses are augmented in mice where Langerin⁺ LCs are selectively deleted (2).

To investigate the potential of immune cells to protect against carcinogenesis, a two-stage cutaneous chemical carcinogenesis model is commonly used wherein single exposure of FVB mouse skin to the “initiator” 7,12-dimethylbenz[*a*]anthracene (DMBA), followed by repeated application of tumor “promoter,” 12-*O*-tetradecanoylphorbol 13-acetate (TPA), induces papillomas, some of which develop into squamous cell carcinomas (SCCs) (4, 5). An activating codon 61 mutation of the *Hras* proto-oncogene within affected keratinocytes characterizes >90% of DMBA-induced SCCs (6). DMBA is representative of mutagenic polyaromatic hydrocarbons (PAHs) to which humans can be exposed, and similar mutations are common in human carcinomas. Although TPA is pleiotropic, its proinflammatory effects are crucial to tumor promotion (7), consistent with common associations of inflammation with carcinogenesis. Thus, two-stage chemical carcinogenesis mimics many molecular and etiological aspects of human cancer. By applying two-stage carcinogenesis to gene-knockout mice, we and others have identified nonredundant host-protective roles of discrete lymphocyte subsets, including epidermal $\gamma\delta$ T cells (1, 5) and cytolytic $\alpha\beta$ T cells (8). Conversely, carcinogenesis may be enhanced by immunosuppressive CD4⁺ “T-reg” (9) and noncytolytic, interleukin (IL)–17—producing CD8⁺ $\alpha\beta$ “T-pro” cells (10).

Unexpectedly, LC-deficient huLangerin–diphtheria toxin A (Lang-DTA) mice showed almost complete resistance to DMBA-TPA-induced cutaneous carcinogenesis (1). Unaware of another example of such a profound cancer-protective effect afforded by removal of a single cell type, we sought to better characterize this finding and investigate the underlying mechanism (11). Because LCs can express T cell suppressive cytokines such as IL-10, we first considered that their absence might confer protection by augmenting antitumor potentials of $\gamma\delta$ and $\alpha\beta$ T cells. However, the marked resistance to carcinogenesis was comparable in Lang-DTA (1) and *Tcr β* ^{−/−} *Tcr δ* ^{−/−} Lang-DTA

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animals (Fig. 1A). Hence, tumor resistance conferred by loss of LCs is T cell-independent. Moreover, resistance was even seen under high-dose protocols in the selective absence of immunoprotective $\gamma\delta$ T cells, a combination shown (5) to induce the highest rate of cutaneous carcinoma (Fig. 1, B and C). This profound impairment of tumor formation on the highly susceptible FVB background suggested that LCs impart an early, requisite influence on keratinocyte transformation. Notably, LCs are located adjacent to both inter- and intra-follicular basal keratinocytes (12), and hair follicle bulge cells have been strongly implicated as targets of cutaneously applied chemical mutagens (13).

K5Hras transgenic mice express a constitutively active HRAS protein within basal keratinocytes (14) and, on the FVB background, show early onset cutaneous SCC (15) (fig. S1A). Crossing the Lang-DTA transgene onto K5Hras transgenic mice did not afford protection from tumor formation (fig. S1, B and C). Because the trans-

genic provision of mutated *Hras* overcame the effect of LC deficiency, we reasoned that generation of cells with *Hras* mutations may ordinarily be fostered by LCs. Therefore, we assessed DNA damage in keratinocytes 24 hours after cutaneous application of DMBA. Phosphorylated histone H2A variant H2AX (γ H2AX), which is recruited to sites of DNA damage, showed more than threefold higher frequency staining within basal keratinocytes of DMBA-exposed skin of normal littermate controls (NLCs) relative to Lang-DTA mice (Fig. 2, A to C, and movie S1). Although some DNA damage was observed in Lang-DTA skin, this level is seemingly insufficient qualitatively and/or quantitatively to overcome the threshold required for tumor development. In contrast, application of the mutagenic DMBA metabolite DMBA-*trans*-3,4-dihydrodiol (DMBA-t-3, 4-diol) induced comparable levels of DNA damage in both strains, implicating LCs in the metabolism of DMBA to its mutagenic forms (Fig. 2C).

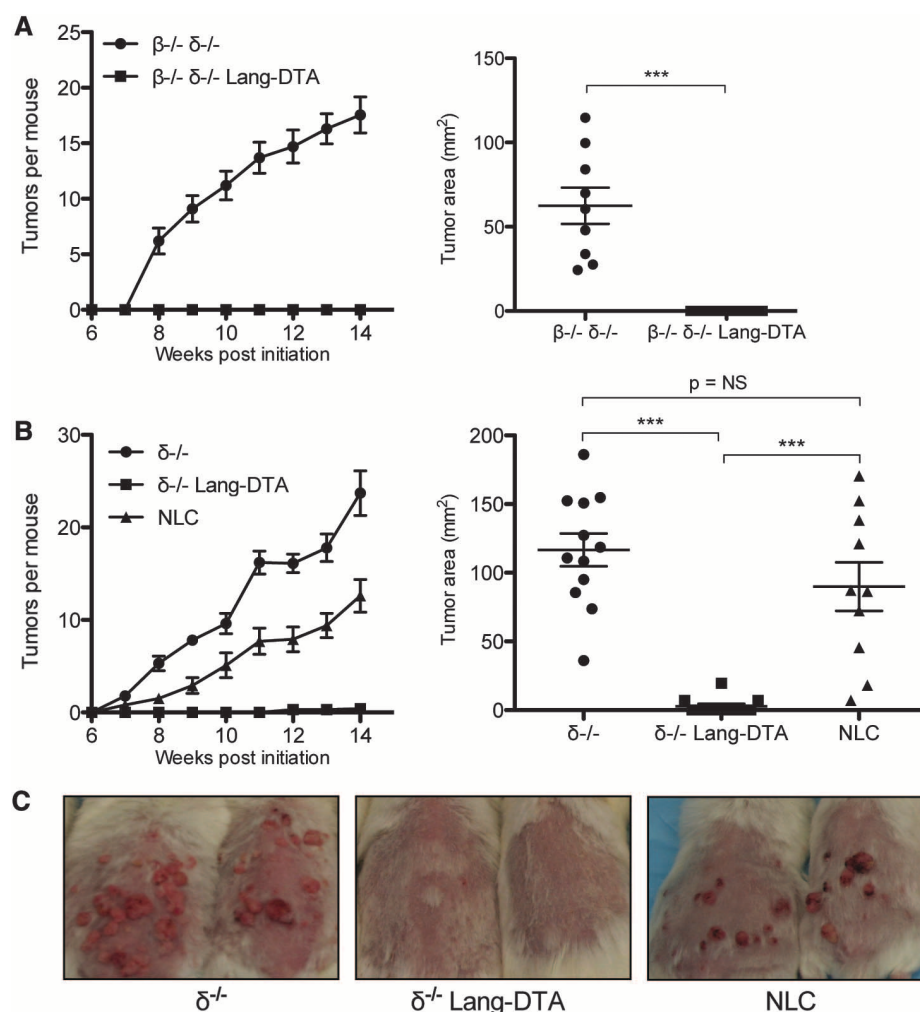


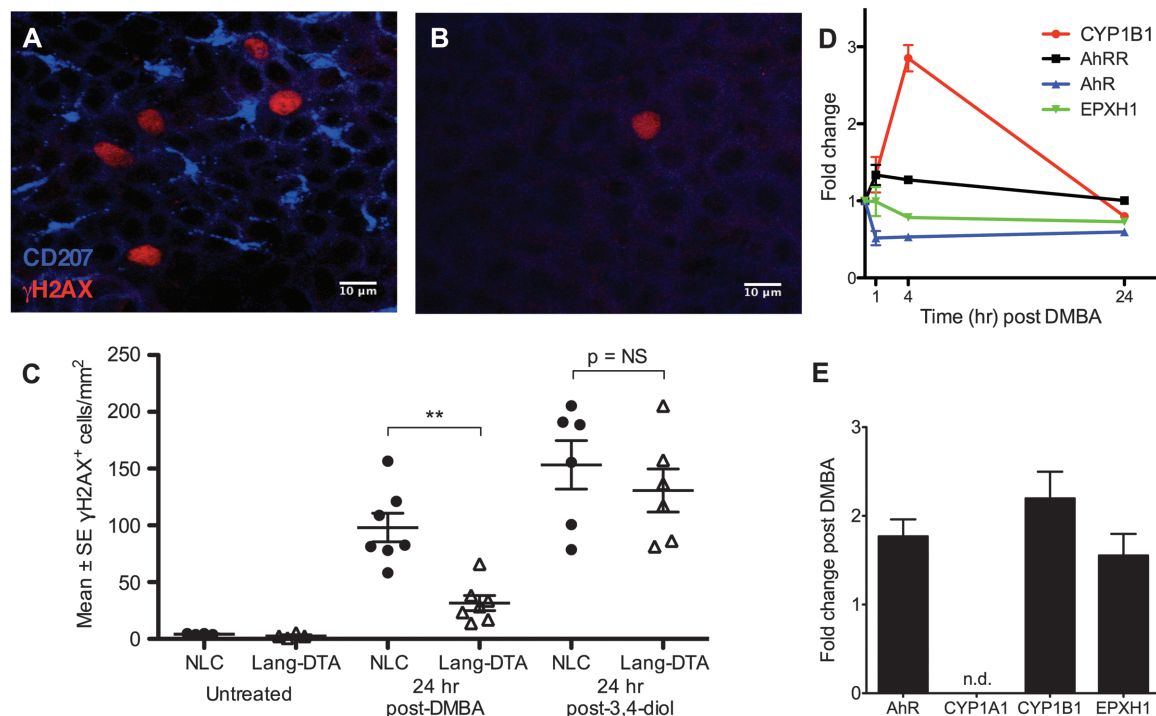
Fig. 1. Resistance to two-stage chemical carcinogenesis is $\alpha\beta$ and $\gamma\delta$ T cell-independent. Chemical carcinogenesis was induced in (A) $Tcr\beta^{-/-} Tcr\delta^{-/-}$ Lang-DTA mice using 50 μ g DMBA to initiate and 6.25 μ g TPA/week to promote, as well as in (B) $Tcr\delta^{-/-}$ Lang-DTA mice using 100 μ g DMBA to initiate and 25 μ g TPA/week to promote. The numbers of tumors per mouse (left) and individual tumor area (right) 14 weeks postinitiation are shown (mean $\pm$ SEM). *** $P < 0.0001$ (Student's t test); $n = 8$ to 12 mice per group. NS, not significant. (C) Representative images from (B).

The carcinogenic potential of DMBA is unleashed by bioactivation via cytochrome P-450 enzymes (e.g., CYP1A1, CYP1B1) and microsomal epoxide hydrolase (EPXH1) after engagement of the aryl hydrocarbon receptor (AHR). In epithelial cells, including keratinocytes and hepatocytes, PAH engagement of the AHR markedly up-regulates the *Cyp1a1*, *Cyp1b1*, and *Epxh1* genes as part of the so-called transcriptional xenobiotic response. Although this response may have evolved for detoxification of potentially harmful natural exogenous aromatic compounds, accumulating evidence suggests roles for AHR in activation and differentiation of cells in response to endogenous ligands, such as the tryptophan photooxidation product 6-formylindolo[3,2-b]carbazole (16). In this regard, AHR has been implicated in LC development, differentiation, and activation (16–18). Murine LCs reportedly do not express *Cyp1a1* (18), however, and when we examined the interaction between DMBA and the Langerin⁺ MHC-II⁺, Birbeck granule⁺ LC line, XS106 (Fig. 2D), or freshly isolated murine epidermal LCs (Fig. 2E), neither baseline nor DMBA-inducible expression of *Cyp1a1* was observed. Rather, primary murine LCs showed modest, transient increases in *Cyp1b1*, *Epxh1*, and the *Ahr* repressor, *Ahr*. This is important because previous studies in vitro using purified recombinant murine enzymes clearly show that metabolism of DMBA to the mutagenic DMBA-t-3,4-diol occurs preferentially via CYP1B1 (19–21), whereas CYP1A1 metabolism is biased toward nonmutagenic detoxification (fig. S2) (22, 23). DMBA-t-3,4-diol is converted to DMBA-3,4-diol-1,2-epoxide (DMBADE), which is immediately proximal to induction of the *Hras* codon 61 “signature” point mutation (24, 25).

To pursue the impact of LCs on *Hras* mutation in vivo, we designed a quantitative genomic DNA (gDNA) polymerase chain reaction (PCR) assay that selectively amplifies the DMBA-induced *Hras* codon 61 mutation (fig. S3A) (26). We generated plasmids containing either wild-type *Hras* (*Hras* WT) or codon 61 mutant *Hras* (*Hras*-mut61) and demonstrated the efficiency of this assay in *Hras*-mut61 quantification (fig. S3B). Using this assay, relative to primary murine keratinocytes (mKC), *Hras*-mut61 was found at levels exceeding 10^3 -fold in the SCC line CarC (100% *Hras*-mut61 mutant) and exceeding 10^2 -fold in skin of DMBA/TPA-treated FVB mice (fig. S3C). Serial dilution of CarC gDNA into aliquots of mKC gDNA showed a capacity to detect one mutant allele against a background of $>10^5$ normal alleles (fig. S3D). When skin was assayed after a single cutaneous DMBA application followed by weekly TPA, significantly fewer *Hras* mutations in the skin of Lang-DTA mice, relative to NLCs, were apparent after only 2 weeks (Fig. 3), approximately 6 weeks before any palpable signs of tumor formation. During the precancerous period, the differential frequency of *Hras*-mut61 in Lang-DTA mice became more apparent over time and was again unaffected by the presence or

Fig. 2. LCs enhance DMBA-induced DNA damage. Confocal microscopy of epidermal sheets isolated from the ears of (A) NLCs or (B) Lang-DTA mice 24 hours post-DMBA application shows LCs (CD207+) in blue and nuclei containing DNA damage (γ H2AX+) in red. (C) The ears of NLCs and Lang-DTA mice were treated with 8.75 μ g DMBA or DMBA-t-3,4-diol and γ H2AX+ cells enumerated 24 hours posttreatment. Each dot represents an individual mouse. ** p < 0.001 (Student's t test). Similar results were obtained from vehicle (acetone)-treated and -untreated mice. (D) XS106 was treated with 64 μ M DMBA and changes in gene expression monitored by quantitative real-time fluorescence

PCR (qPCR). *Cyp1a1* expression is not shown because it was undetectable at all time points. (E) NLC mice were untreated or treated with 100 μ g DMBA, and, 24 hours later, epidermal Langerhans cells were purified by cell sorting and



analyzed for the expression of the indicated genes by qPCR. Purified LCs did not express *Cyp1a1* either before or after DMBA treatment (n.d., not detected). All graphs depict mean $\pm$ SEM.

absence of T cells (Fig. 3). High-throughput next-generation sequencing (Illumina HiSeq 2000) likewise demonstrated the accumulation of substantially more *Hras* codon 61 mutations in DMBA-treated LC-intact skin versus LC-deficient skin (fig. S4). Taking the data together, we hypothesized that the very high CYP1B1:CYP1A1 ratio in LCs favors metabolism of DMBA to mutagenic DMBA-t-3,4-diol that then may be available for subsequent conversion to mutagenic DMBADe within neighboring keratinocytes.

To test the potential of LCs to metabolize DMBA, we analyzed lysates of XS106 cells 24 hours post-DMBA exposure by high-performance liquid chromatography (HPLC). Internalization of DMBA was evidenced by a single spike (Fig. 4A), whereas the supernatant from such cultures markedly revealed an additional cluster of spikes, consistent with DMBA metabolites (Fig. 4B). The concentration of XS106-internalized DMBA increased with increasing initial DMBA concentrations (Fig. 4C). Notably, there was a very rapid accumulation of DMBA metabolites in the medium of XS106 cells, which also increased over time and with the concentration of DMBA (Fig. 4, D and E). These data are consistent with the cells' uptake and metabolism of DMBA and the rapid release of metabolites. Liquid chromatography/tandem mass spectrometry (LC/MS/MS) readily identified the mutagenic metabolite DMBA-t-3,4-diol in the cultures (Fig. 4F) at mean concentrations of 29.2 nM in 32 μ M-DMBA-exposed XS106 samples and 42.4 nM in 64 μ M-DMBA-

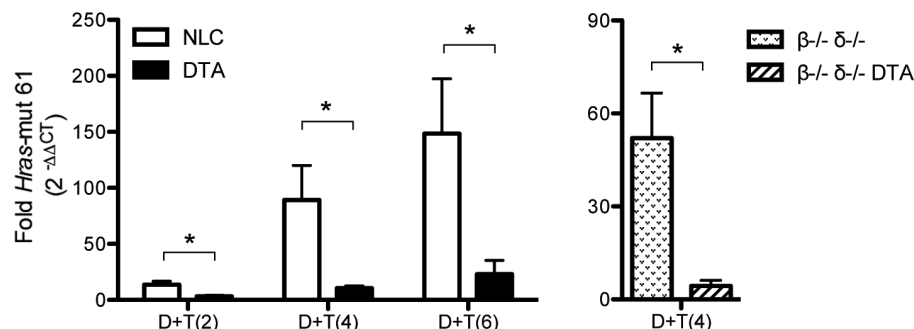


Fig. 3. Reduced levels of cutaneous *Hras* codon 61 mutations in LC-deficient mice detected by qPCR of gDNA. Lang-DTA, *Tcr $\beta^{-/-}$ Tcr $\delta^{-/-}$* Lang-DTA, and appropriate NLC animals were treated with DMBA [200 μ g for D+T(2), 100 μ g for all others], followed by TPA (25 μ g per week, number of weeks in parenthesis). *Hras* codon 61 mutations in the treated skin were quantified by qPCR of gDNA. * P < 0.01 (Student's t test), n = 5 to 6 mice per group. Error bars indicate SEM.

exposed samples. No DMBA-t-3,4-diol was detected in cultures lacking either XS106 or DMBA. These data evoke another study which showed that the LC line, XS52, could metabolize the PAH benzo[*a*]pyrene, to diol, quinone, and phenol metabolites (27). To further test the hypothesis that the profound contribution of LCs to carcinogenesis reflects the cells' release of mutagens formed by uptake and metabolism of DMBA, we applied synthesized DMBA-t-3,4-diol as the initiating agent in two-stage carcinogenesis of Lang-DTA and NLC mice. In contrast to the markedly reduced tumorigenesis observed in DMBA-treated Lang-DTA mice, DMBA-t-3,4-diol initiated comparable tu-

mor development in the two strains (Fig. 4G). Thus, by artificially providing a mutagenic metabolite of DMBA, the substantial contribution of LCs to carcinogenesis was bypassed.

Given the implication that murine LCs contribute to cutaneous chemical carcinogenesis via nonimmunological properties, it was appropriate to determine whether human LCs might display equivalent properties with the potential to promote disease. We therefore examined the response to DMBA of primary human LCs from several donors and their consequent effects on primary keratinocytes. The data were consistent with the murine data sets. After DMBA treatment, human

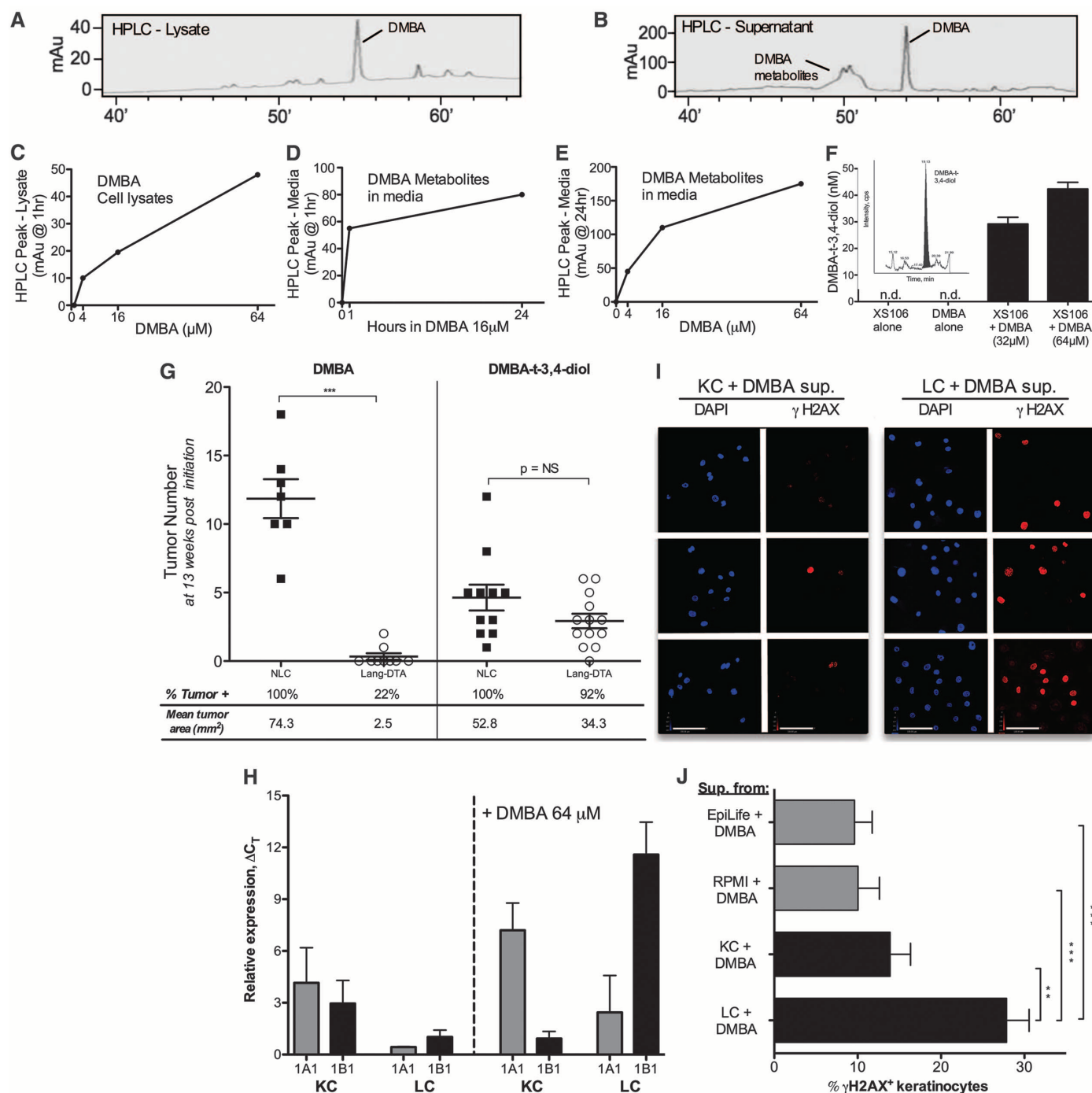


Fig. 4. LCs have the capacity to metabolize DMBA to DMBA-t,3,4-diol, and initiation with this metabolite abrogates the tumor resistance seen in LC-deficient mice. XS106 cells were cultured with 64 μ M DMBA for 24 hours, the supernatant was then collected, and cells were pelleted to prepare a lysate. (A and C) HPLC analysis of the lysate. (B, D, and E) HPLC analysis of the supernatant. (F) LC/MS/MS analysis of XS106 cells in the presence or absence of DMBA. (G) Tumor induction after treatment with DMBA (12.5 μ g) or DMBA-t,3,4-diol (100 μ g). 25 μ g/week TPA promotion was used in both groups, and initiator doses were chosen on the basis of predicted diol instability, reduced cutaneous penetrance, and pre-

liminary studies. Each dot represents an individual mouse. (H) Human keratinocytes or LCs were cultured in the presence (right) or absence (left) of 64 μ M DMBA for 24 hours, then *Cyp1a1* (1A1) and *Cyp1b1* (1B1) expression was quantified relative to β -actin by qPCR. (I and J) Human keratinocytes (in EpiLife medium) or LCs [in RPMI^{wt(-)} medium] were cultured 24 hours in the presence (or absence, not shown here) of 64 μ M DMBA. Supernatants (sup.) were then harvested and transferred onto fresh keratinocyte cultures for an additional 24 hours, then DNA damage was assessed by γ H2AX staining. Confocal microscopy (I) and quantification of the data (J). ** P < 0.001, *** P < 0.0001 (Student's t test).

LCs expressed substantially more *Cyp1b1* than *Cyp1a1* (of which most donors' cells express negligible levels), whereas human keratinocytes expressed an excess of *Cyp1a1* (Fig. 4H). For most donors, *Cyp1b1* expression by DMBA-treated

LCs was ~20-fold higher than that in keratinocytes, whereas *Cyp1a1* expression was substantially higher in keratinocytes. To determine whether human LCs are more proficient than keratinocytes at metabolizing DMBA to a mutagenic

product(s) that they then export, primary human LCs or keratinocytes were cultured with DMBA for 24 hours, and supernatants were harvested and then transferred onto new primary keratinocyte cultures. After an additional 24-hour incu-

bation, the keratinocytes were assayed for DNA damage by γ H2AX staining. Because keratinocytes were grown in different media (EpiLife) than LCs [tryptophan-deficient RPMI: RPMI^{W(-)}], controls included each medium cultured with DMBA for 24 hours (but without LCs or keratinocytes present). The supernatants from DMBA-treated LCs, but not those from identically treated keratinocytes, provoked a significant increase in DNA damage above baseline (Fig. 4, I and J). Hence, although DNA damage can result from the cell-autonomous breakdown of DMBA by keratinocytes, it is substantially increased by the actions in trans of human LCs.

LCs survey the epidermis via both locomotion and repetitive extension/contraction of their dendritic processes and migrate to draining lymph nodes where their main function has been viewed as initiating and/or regulating adaptive immune responses (28). Here, however, a major pathophysiologic role for LCs is described that is independent of adaptive immunity. Instead, it highlights the tissue-scavenging functions of LCs, by which they take up and metabolize chemical contaminants of the epidermis. Although this capability may powerfully attenuate the potency of natural toxins, it may be confounded by industrial PAHs such as DMBA where the detoxified metabolites that are released are more mutagenic than the starting compound. Thus, this innate action of LCs increases DNA damage and specific *Hras* mutations in neighboring keratinocytes. Although further studies are necessary to determine the precise mechanism by which LCs transfer DMBA metabolites to keratinocytes, the proximity of LCs to basal keratinocytes is evident. Given that PAHs are highly prevalent in industrial pollution and that extracts of airborne particles topically applied to mouse skin results in SCC development that is *Ahr* dependent (29), PAH-containing particulate matter might represent an underappreciated environmental factor in human skin cancer. Activating *Ras* mutations are found in ~50% of human epidermal SCCs (30), and in xenografting experiments, the activation of *Hras* signaling (plus inhibition of NF- κ B) was entirely sufficient to transform primary human keratinocytes into SCCs (31).

Although the capacity of keratinocytes to metabolize DMBA (32) and express CYP1A1, CYP1B1, and EPXH1 enzymes (33) has clearly been demonstrated previously, this is nonetheless insufficient to induce substantial tumor formation in the absence of LCs. Others have revealed the potential for nonepithelial stromal cells to activate PAH mutagens (34). The marked resistance of LC-deficient skin to chemical carcinogenesis, in an experimental system optimized for tumor formation, markedly establishes the capacity of LCs to substantially enhance the toxicity of environmental agents. Collectively, our data are consistent with a cooperative carcinogenicity scenario in which LC CYP1B1 and EPXH1 preferentially metabolize DMBA to DMBA-t-3,4-diol, which is subsequently delivered to adjacent keratinocytes wherein CYP1A1 converts the DMBA-t-3,4-diol

to mutagenic DMBAD (fig. S2). Furthermore, our findings also provoke the possibility that locally resident DC populations may enhance PAH-induced mutations and tumor development within other epithelial tissues, contributing to the risk of lung, colon, and genitourinary carcinomas.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/335/6064/104/DC1
Materials and Methods
Figs. S1 to S4
References (35–37)
Movie S1

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Stop Signals Provide Cross Inhibition in Collective Decision-Making by Honeybee Swarms

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Honeybee swarms and complex brains show many parallels in how they make decisions. In both, separate populations of units (bees or neurons) integrate noisy evidence for alternatives, and, when one population exceeds a threshold, the alternative it represents is chosen. We show that a key feature of a brain—cross inhibition between the evidence-accumulating populations—also exists in a swarm as it chooses its nesting site. Nest-site scouts send inhibitory stop signals to other scouts producing waggle dances, causing them to cease dancing, and each scout targets scouts' reporting sites other than her own. An analytic model shows that cross inhibition between populations of scout bees increases the reliability of swarm decision-making by solving the problem of deadlock over equal sites.

The decision-making mechanisms in nervous systems and insect societies are quite similar (1–3). In both types of systems, the decision-making process is a competition

between mutually interacting populations of excitable units (neurons or individuals) that accumulate noisy evidence for alternatives, and, when one population exceeds a threshold level of activ-

ity, the corresponding alternative is chosen (4–7). An important feature of many of the models of neural decision-making is that each population of integrator neurons inhibits the activation of the others to a degree proportional to its own activation (8–10). These inhibitory connections help ensure that only one of the alternatives is chosen and may enable statistically optimal decision-making (3, 10). Given the importance of cross inhibition in neural decision-making circuits, we looked for cross inhibition in the social decision-making process of a honeybee swarm choosing a nest site.

Honeybee swarms are produced in the spring when several thousand worker bees leave their hive with their mother queen to establish a new colony. The swarming bees cluster near the parental hive for a few days while several hundred of the oldest bees in the swarm, the scout bees, find prospective nest sites and choose the best one (11, 12). We began our study with the finding that scout bees use a signal for the inhibition of waggle dances—the stop signal—during the process of choosing their swarm’s future home. We then observed swarms choosing between two nest boxes and found that the scout bees committed to each box directed their stop signals mainly toward scouts promoting the other box; this created cross inhibition between the two populations of scout bees. Lastly, we explored the functional implications of this cross-inhibitory signaling by modeling the bees’ collective decision-making process.

Honeybees possess an inhibitory signal, the stop signal, that is known to reduce waggle dancing and recruitment of foragers to food sources (13–15). Bees that have been attacked while foraging produce stop signals upon return to the hive, preferentially targeting nestmates visiting the same food source (16). The stop signal is a vibrational signal that lasts about 150 ms, has a fundamental frequency around 350 Hz (17), and is typically delivered by the sender butting her head against the dancer (13). Dancers usually do not show an immediate response to a stop signal. Rather, an accumulation of stop signals increases the probability that a bee will cease dancing. The stop signal enables a colony to reduce its recruitment to food sources that are perilous (16).

Knowing that foraging bees use the stop signal to inhibit waggle dances advertising food sources, we explored whether house-hunting bees use this signal to inhibit dances advertising nest sites. We began by making video and sound recordings of nest-site scouts performing waggle dances on five swarms (18). Close-up recordings of 40 dancers on two of these swarms (20 dancers

per swarm) revealed the use of stop signals. These bees produced dances that lasted 74 ± 54 s (mean $\pm$ SD) and contained 24 ± 20 dance circuits, and 23 of these dancing bees received a total of 109 stop signals [(Fig. 1); 4.7 ± 4.3 signals per signaled bee] that were produced by 40 different bees. Each bee that produced a stop signal followed a dancer for 3.0 ± 1.5 dance circuits before lunging toward the dancer, contacting her with head (98%) or thorax (2%) for 0.25 ± 0.44 s, and delivering 2.4 ± 1.9 stop signals, each of which lasted 0.21 ± 0.10 s. Senders disproportionately contacted dancers during the return phase of the dance (96 contacts) rather than the waggle phase (13 contacts) [if delivered in proportion to the lengths of these phases, 73.0 and 36.0 contacts would be expected; chi-squared test, $\chi^2(1, 109) = 21.9$, $P < 0.0001$]. Dancing bees that received stop signals ceased dancing shortly (36 ± 22 s) after they began to receive the signals. After ceasing to dance, 17 of the 23 bees walked quietly over the swarm cluster, 3 started to produce piping signals to stimulate others to warm up for departure (19), and 3 flew off.

To clarify the relation between receiving stop signals and stopping dancing, we recorded 109 dances on three more swarms and determined the distribution of the 525 stop signals received during these dances. Stop signals occurred more toward the ends of dances than expected if these signals had been given at random [(Fig. 1C); chi-squared test, $\chi^2(9, 525) = 234$, $P < 0.0001$ (all dances); $\chi^2(9, 358) = 58$, $P < 0.0001$ (long dances)]. Evidently, the stop signals caused the cessation of dancing. If the relation were not causal but instead were a result of stop signals and ends-of-dances both becoming more likely as dances progress, then longer dances should have begun receiving many stop signals midway through. Yet even in the long dances the stop signals were strongly overrepresented in the last circuits. It is likely that dances ended because

stop-signal inhibition exceeded some threshold in the final circuits.

We next conducted an experimental study to determine how bees use the stop signal throughout a swarm’s process of choosing its nest site. We set up two swarms, one at a time, on an island devoid of natural nesting cavities and provided them with a choice of two identical nest boxes. Scout bees visiting the nest boxes were labeled with nest-box-specific paint marks (pink or yellow). We recorded video of the scouts producing waggle dances and tracked them (one at a time) with a microphone to know when they received stop signals. In most cases (98.4%, $n = 1379$), we could identify which bee produced a given stop signal; each time we heard one, we noted which bee standing near the dancer lunged toward and pressed against her. Nearly every stop signal (94.5%, $n = 1357$) was produced by a bee bearing a paint mark, hence, by a nest-site scout.

There were notable differences in the colors of the paint marks of the scout bees delivering stop signals toward the dancers for the two nest boxes during the decision phase of the nest-site selection process, that is, when a swarm is choosing among possible nest sites. In both swarms, the pink and yellow dancers both received many more signals from different-colored bees (“contra signals”) than from same-colored bees (“ipsi signals”): contra signals, 213, from at least 46 bees; ipsi signals, 24, from at least 14 bees. Moreover, both pink and yellow dancers received disproportionately more contra signals than would be expected if the signals had been delivered in proportion to the number of scouts of each color marked at the times of their dances [Fig. 2; chi-squared test, $\chi^2(1, 237) = 114.55$, $P < 0.0001$ (analysis of signals); $\chi^2(1, 60) = 16.18$, $P < 0.0001$ (analysis of minimum signalers); see (18) for explanation of the two statistical analyses]. How the scout bees discriminated the two types of dancers is not known. They may have decoded the location of

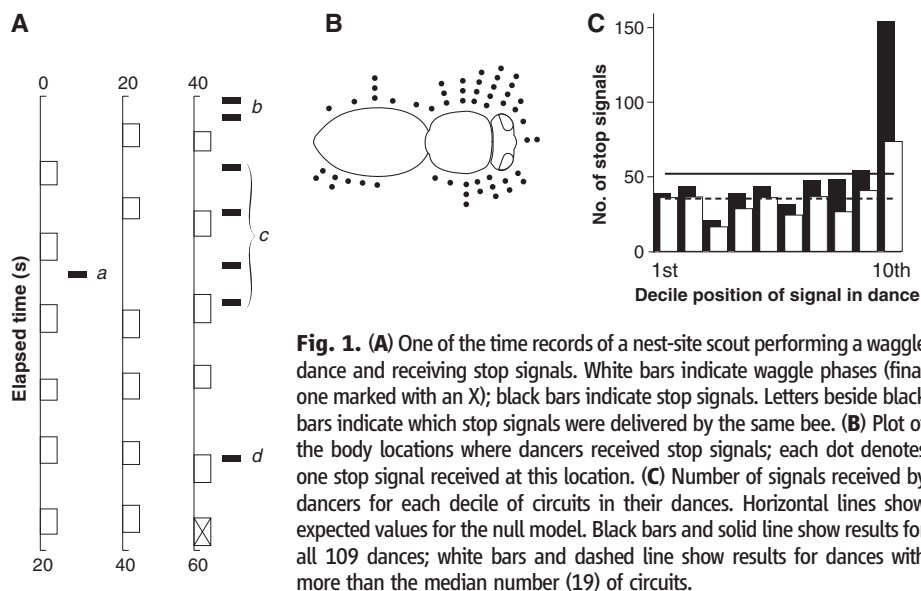


Fig. 1. (A) One of the time records of a nest-site scout performing a waggle dance and receiving stop signals. White bars indicate waggle phases (final one marked with an X); black bars indicate stop signals. Letters beside black bars indicate which stop signals were delivered by the same bee. (B) Plot of the body locations where dancers received stop signals; each dot denotes one stop signal received at this location. (C) Number of signals received by dancers for each decade of circuits in their dances. Horizontal lines show expected values for the null model. Black bars and solid line show results for all 109 dances; white bars and dashed line show results for dances with more than the median number (19) of circuits.

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each dance or—as is the case for stop-signaling bees in the context of foraging (13)—they may have used odor differences. Because stop signals were delivered to unmarked bees in the first five swarms studied, visual cues from the paint marks were most likely not involved.

During the implementation phase, that is, when a swarm has finished choosing its nest site and is preparing to move there, the scout bees no longer directed their stop signals primarily at dancers advertising the other site. In both swarms, pink and yellow dancers received contra signals (185 from at least 70 signalers) and ipsi signals (210 from at least 79 signalers) in proportion to the number of different-colored scouts and same-colored scouts marked at the time of their dances [chi-squared test, $\chi^2(1, 395) = 1.49$, $P < 0.23$ (analysis of signals); $\chi^2(1, 149) = 0.34$, $P < 0.56$ (analysis of minimum signalers)].

Thus, in both swarms there was evidence of cross inhibition between the two groups of scout bees when these groups were competing to reach a threshold (quorum). Once one group did so, indicated by the onset of worker piping (19), there was a general inhibition of waggle dancing. Shutting down recruitment during the implementation phase through stop signaling helps ensure that all the scout bees will be present on the cluster when the swarm flies to the chosen site.

This interpretation of the results of the two-nest-boxes trials is confirmed by the results from two additional swarms that made a “choice” with only one nest box under consideration. If stop signals function mainly for cross inhibition of waggle dances in the decision phase and for general inhibition of dances in the implementation phase, then in the decision phase the proportion of dancers receiving stop signals should be smaller in the one-nest-box trials than in the two-nest-boxes trials. In the implementation phase, however, the proportion should not be smaller in the one-nest-box trials. These are precisely the patterns that we found. In the decision phase, only 26% of dancers ($n = 38$) received stop signals when one nest box was under consideration, whereas 66% ($n = 74$) did so when two boxes were [Fig. 2; chi-squared test, $\chi^2(1, 112) = 16.03$, $P < 0.0001$]. In the implementation phase, the respective percentages are 86% ($n = 148$) and 77% ($n = 133$) (chi-squared test, $\chi^2(1, 281) = 3.92$, $P = 0.05$).

We have demonstrated that the stop signal is an integral part of the decision-making process used by a honeybee swarm to choose its nest site. During the initial phase of the process, when the choice is being made, this signal creates cross inhibition between populations of scout bees representing different sites. This cross inhibition curtails the production of waggle dances for, and thus the recruitment of bees to, a competing site. Because we know from previous studies (20, 21) that when a scout bee stops producing waggle dances for a site she soon stops making visits to the site, we can be confident that the cross inhibition created with the stop signal also inhibits

the number of bees visiting a competing site. Thus, it appears that the stop signals in bee swarms serve the same purpose as the inhibitory connections in models of decision-making in primate brains, such as the Usher-McClelland (U-M) model: to suppress the activity levels of integrators representing different alternatives (3, 8).

The similarities between the decision-making processes in honeybee swarms and in the U-M model are notable. In both, there are populations of units (bees or neurons) that act as mutually inhibitory, leaky integrators of incoming evidence, and in both the choice is made when the integrated evidence supporting one of the alternatives exceeds a threshold (12). To understand the implications of the observed stop-signaling behavior, we have modeled the collective decision-making process of the bees by using ordinary differential equations rigorously derived from the individual-

level interaction rules that we have determined through empirical observations (18). These equations enabled us to analytically describe the average population-level behavior of the scout bees over time. The models of decision-making that we analyzed in this manner were (i) a model proposed to enable statistically optimal collective decision-making through individual bees inducing each other to directly change their commitment (3), (ii) a model based on the observed stop-signaling behavior of honeybees but assuming that stop signals are delivered indiscriminately to all bees encountered, and (iii) a model based on the observed stop-signaling behavior but including the observation that stop signals are largely delivered to bees dancing for a site that differs from the one the signaler has encountered.

The results of this modeling work, illustrated in Fig. 3 and presented in detail in (18), are

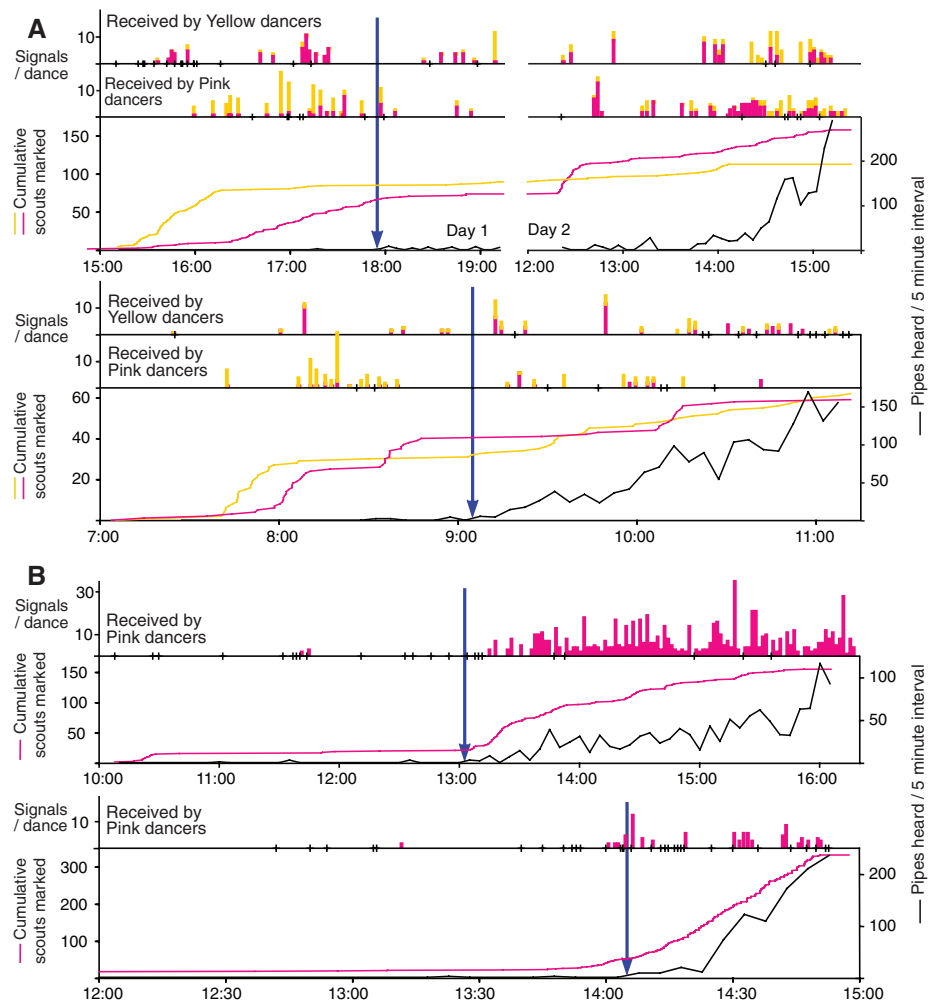


Fig. 2. Patterns of stop signaling and of growth in the populations of scout bees with paint marks throughout the nest-site selection processes of four swarms. In the upper portion of each graph, each dance observed is represented by a tic if it received no stop signals or a colored bar indicating the number of stop signals received from scouts marked with each color (rare unmarked signalers are not shown). A vertical arrow indicates the onset of worker piping, the acoustic signal produced by scouts to stimulate the nonscouts in a swarm to prepare to fly to the chosen site (19). Hence, each vertical arrow marks the transition from making to implementing the swarm's choice. (A and B) Results for trials with two nest boxes or one nest box, respectively.

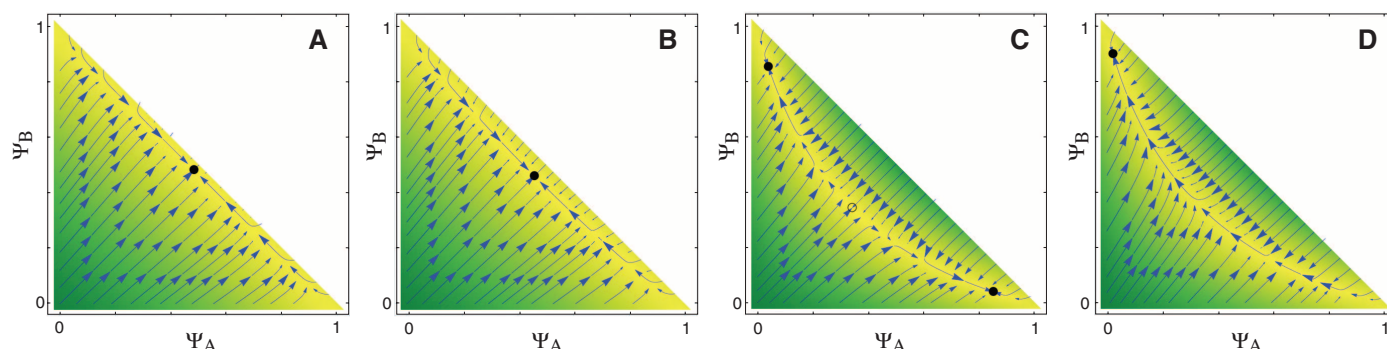


Fig. 3. Dynamical behavior of the different models described in the text, demonstrating how discriminate cross inhibition enhances the decision-making reliability of a honeybee swarm. The two axes show the proportions of a swarm's scout bees, Ψ_A and Ψ_B , that are committed to two alternative nest sites, A and B, respectively. The directions and lengths of arrows illustrate net change in these proportions over a fixed time interval and so represent the dynamical behavior of the models. Stable and unstable fixed points are shown as solid and open black circles, respectively. **(A)** The direct-switching model of (3) with finite decay and equal alternatives, showing convergence to stable

deadlock between the available sites. **(B)** The discriminate stop-signaling model with equal alternatives but with incidence of stop signaling below the critical threshold, so that the stable deadlock seen in **(A)** persists. **(C)** The model shown in **(B)**, but with the incidence of stop signaling above the critical threshold; as a result, the swarm randomly achieves a consensus for one of the two equal alternatives. **(D)** The model shown in **(B)** and **(C)**, but with the difference in the qualities of the two alternatives exceeding a critical threshold; as a result, the swarm is expected to converge on a consensus for the superior alternative.

conclusive. They show that, for the first model (i), given just the tiniest amount of decay from the integrating populations of scouts (probably inescapable in a real biological system), a decision between two equal alternatives (as studied empirically in this paper) inevitably results in a stable deadlock with equal numbers of scouts committed to the two alternatives. When one site gains a majority of scouts, the switching of scouts from it to the other site increases, forcing the system back to a state of equal commitment [Fig. 3A and (18)]. Such stable deadlock is clearly sub-optimal, because it will result in the swarm never achieving a consensus; this could mean the swarm never lifts off. If it does, the equal numbers of scouts committed to two different sites will cause problems for the swarm's cohesiveness as it attempts to fly to its new home (22, 23). The same situation occurs for the indiscriminate stop-signal model (ii) when the alternatives are equal (18). Stable deadlock is also observed for the discriminate stop-signaling model (iii) when the incidence of stop signaling is below a critical threshold (Fig. 3B). Once that threshold is exceeded, however, two stable attractors appear, one for each nest site, and the swarm chooses at random between the two equal alternatives [Fig. 3C and (18)]. Such signaling behavior thus breaks the deadlock of the previous models and allows the swarm to quickly converge on a consensus decision. Importantly, this discriminate stop-signaling model is in accordance with experimental observations (Fig. 2). Intriguingly, when the difference in the qualities of the two alternatives exceeds a critical

threshold, the swarm is expected to converge on the better of the two options [Fig. 3D and (18)].

For neural models of decision-making, cross inhibition between integrating populations is crucial for effective decision-making and has been shown to allow optimal decisions under some circumstances (10, 24). As we have shown here, cross inhibition between integrating populations is also present in honeybee swarms and is very important for their success when making decisions. It is tempting to think that the ability to implement a highly reliable strategy of decision-making is what underlies the astonishing convergence in the functional organization of these two distinct forms of decision-making system: a brain built of neurons and a swarm built of bees.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1210361/DC1
Materials and Methods
SOM Text
Figs. S1 to S4
Tables S1 and S2
References (25–44)
Movie S1

27 June 2011; accepted 21 November 2011
Published online 8 December 2011;
10.1126/science.1210361

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Nina V. Fedoroff

AAAS President and 2012 Program Chair

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The Annual Meeting is one of the most widely recognized global science events with hundreds of networking opportunities and broad global media coverage. An exceptional array of speakers will gather from 16-20 February in Vancouver, BC. The theme of "Flattening the World: Building a Global Knowledge Society" is intended to focus the program on the complex, interconnected challenges of the 21st century and on pathways to global solutions through international, multidisciplinary efforts.

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AAAS last convened outside the United States in 1981 in Toronto. We will meet in Vancouver in 2012 for the first time. The science and technology community in Canada has provided invaluable assistance with our planning efforts, which I gratefully acknowledge. Our sections, divisions, and committees also make tremendous contributions, which are integral to the program. I also extend a personal thanks to the members of the Scientific Program Committee who organize the many excellent ideas and contributed proposals submitted into an outstanding meeting.

I urge you to join us in Vancouver.

Nina V. Fedoroff

AAAS President and Program Chair;
Evan Pugh Professor, Huck Institutes of the
Life Sciences, Penn State University;
Distinguished Visiting Professor,
King Abdullah University of Science and Technology

President's Address

Thursday, 16 February



Nina V. Fedoroff
Distinguished Visiting
Professor, King Abdullah
University of Science and
Technology, and Evan Pugh
Professor, Huck Institutes
of the Life Sciences,

Pennsylvania State University

Dr. Fedoroff is a leading geneticist and molecular biologist who has contributed to the development of modern techniques used to study and modify plants. Under the administrations of Presidents George Bush and Barack Obama, she served as Science and Technology Adviser to the Secretary of State and to the Administrator of the U.S. Agency for International Development. She is a member of the U.S. National Academy of Sciences, the American Academy of Arts and Sciences, and the Phi Beta Kappa and Sigma Xi honorary societies. Her memberships include the Board of Trustees of the Library of Alexandria, Egypt, and the Board of Governors at the Institute for Complex Adaptive Matter of the Los Alamos Laboratory.

Plenary Speakers

Friday, 17 February



Mike Lazaridis
President and Co-Chief
Executive Officer, Research
In Motion (RIM); Founder
and Board Chair, Perimeter
Institute for Theoretical
Physics

The Power of Ideas

Mr. Lazaridis is known in the global wireless community as a visionary, innovator, and engineer of extraordinary talent. He traces his passion for his ideas and hard work to his boyhood home of Windsor, Ontario, Canada, where his love of science and fascination with electronics were nurtured in supportive family and school environments. While a student in Waterloo, Ontario, an entrepreneurial community that has emerged into a high-tech hotbed and knowledge economy focal point, he founded RIM, launching the smart phone phenomenon with the BlackBerry. Today he is responsible for product strategy, research and development, product development,

and manufacturing. Mr. Lazaridis, born in Istanbul, is also a passionate advocate for education and scientific research. He supports his community, province, and country through his many insights, global perspective, and his generous philanthropic gifts made possible by success in business.

Saturday, 18 February

Plenary Panel

Science Is Not Enough

An exceptional plenary panel will arm scientists, educators, and students with finely worded messages to influence public perceptions and debate about science-related global challenges.



James Hansen
Head of the NASA Goddard
Institute for Space Studies
known for raising broad
awareness of the global
warming issue.



Olivia Judson
An evolutionary biologist
and journalist who often
writes about the influence
of science and biology on
modern life.



Hans Rosling
A global health professor at
the Karolinska Institute and
Gapminder's co-founder
who dispels myths about
the developing world.



Frank Sesno
Moderator

The panel will be moderated by Frank Sesno, an award-winning American journalist, former CNN correspondent, anchor and Washington bureau chief, and director of the School of Media and Public Affairs at George Washington University.

Sunday, 19 February



Ismail Serageldin
Director, The New Library of
Alexandria

Science and Democracy

Dr. Serageldin, an Egyptian national, has advocated for

greater equality in science and society at large, and has been hailed as a champion for using science in sustainable development and for liberating minds from the tyranny of intolerance, bigotry, and fear. Throughout his illustrious career, Serageldin has earned a reputation for applying science to nearly every type of global problem. He is perhaps most highly regarded for his attempts to combat hunger in developing countries through the promotion of sustainable agriculture. Today, he is the director of the New Library of Alexandria, a major cultural and intellectual center built in 2002 near where the famed Library of Alexandria stood from 288 B.C.E. until approximately 400 C.E. when it was destroyed. Serageldin played a leading role in founding the new library, hoping that the institution would rekindle the great intellectual heritage that had defined the Arab and Muslim world.

Monday, 20 February



Frans B.M. de Waal
C. H. Candler Professor of
Psychology, and Director,
Living Links Center, Yerkes
Primate Center, Emory
University

Good Natured: From Primate Social Instincts to Human Morality

Dr. de Waal is a Dutch American behavioral biologist known for his work on the social intelligence of primates. His first book, *Chimpanzee Politics*, compared the schmoozing and scheming of chimpanzees involved in power struggles with that of human politicians. Ever since, de Waal has drawn parallels between primate and human behavior, from peacemaking and morality to culture. His latest book is *The Age of Empathy: Nature's Lessons for a Kinder Society*. He is a member of the U.S. National Academy of Sciences, the American Academy of Arts and Sciences, and the Royal Dutch Academy of Sciences. In 2007, *Time* selected him as one of the Worlds' 100 Most Influential People Today.

Topical Lecture Series



Karen Bakker

Professor of Geography and Director, Program on Water Governance, University of British Columbia; Canada Research Chair in Political Ecology

Water Privatization, Urbanization, and Development



Donald B. Dingwell

Secretary General, European Research Council
Explosive Volcanism in the Earth System



Lillian Eva Dyck

Senator, Parliament of Canada; Professor Emerita, University of Saskatchewan; Member of the Gordon First Nation in Saskatchewan
The Medicine Wheel and Western Science



Michael Hayden

Professor of Medical Genetics; Director, Center for Molecular Medicine and Therapeutics, University of British Columbia; Canada Research Chair in Human

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Distinguished Project Professor, Departments of Cell Biology and Anatomy, and Molecular Structure and Dynamics, University of Tokyo
Kinesin Superfamily

Molecular Motors: From Intracellular Transport to Brain Development, Wiring, and Learning and Memory



Steve MacLean

President, Canadian Space Agency
International S&T Collaboration



Julio Montaner

Professor and Head of Division of AIDS, University of British Columbia; Director of the BC Centre for Excellence in HIV/AIDS, Providence Health Care

Toward the Control of HIV and AIDS: Comprehensive Treatment as Prevention



Yolanda Moses

Professor, University of California, Riverside
Origins of Social Inequality in Complex Societies



Carl Wieman

Associate Director for Science, Office of Science and Technology Policy, U.S. White House
A Scientific Approach to Science Education

JOHN P. MCGOVERN LECTURE IN THE BEHAVIORAL SCIENCES



Joseph LeDoux

Henry and Lucy Moses Professor of Science and University Professor, Center for Neural Science, New York University; Director, Center for the

Neuroscience of Fear and Anxiety
The Emotional Brain

GEORGE SARTON MEMORIAL LECTURE IN THE HISTORY AND PHILOSOPHY OF SCIENCE



Robert Smith

Professor of History and Classics, University of Alberta
Making Science Big: From Little Science to Megaprojects?

Seminars

Friday, 17 February

Unlocking Biology's Potential

Biology research addresses timely topics of deep impact, permeating almost every

aspect of global development, including natural processes that affect food, water, energy, the environment, and health.

Can Proteomics Fill the Gap Between the Genome and Phenotypes?

Organized by Christopher Mark Overall, University of British Columbia; Gilbert S. Omenn, University of Michigan, Ann Arbor

SPEAKERS

Christopher Mark Overall, University of British Columbia

Travelling to the Ends of the Proteome World with Terminomics and Degradomics

Andrew Emili, University of Toronto

A Hidden Connection Is Stronger than an Obvious Connection in the Proteome World

Pascale Gaudet, Swiss Institute of Bioinformatics

The Next Big Thing: Bringing the Human Proteome Project to the World

Delivering on the Promise of the Human Genome Project

Organized by William T. Beck, University of Illinois, Chicago; M. Guill Wientjes, Ohio State University

SPEAKERS

Daniel D. von Hoff, Translational Genomics Research Institute

The Human Genome Project 11 Years Later: New Medical Treatments for Humanity

Anil K. Sood, MD Anderson Cancer Center

Small Interfering RNA: Promises for Treatment, Problems in Delivery

Patrick S. Stayton, University of Washington
siRNA Delivery Through Advances in Polymer Carriers and Nanotechnology

Predictive Medicine

Organized by Richard C. Willson, University of Houston; Russell Lefevre, IEEE-USA

SPEAKERS

Larry Gold, University of Colorado and Somologic

Unlocking Biomarker Discovery

Michael R. Hayden, University of British Columbia, Vancouver

The Experimental Man

John Haynes, National Aeronautics and Space Administration (NASA)

NASA's Use of Remote Sensing To Enable Predictive Health

Seeing Biosphere's Dark Matter: Genomic Methods on Unculturable Microbial Diversity

Organized by Claudio Slamovits, Dalhousie University; Patrick J. Keeling, University of British Columbia

SPEAKERS

Alexandra Z. Worden, Monterey Bay Aquarium Research Institute

Activity, Diversity, and Genetic Make-Up of Wild Marine Photosynthetic Eukaryotes

Patrick J. Keeling, University of British Columbia

Bringing Molecular and Genomic Data to the Uncultivable Masses

Forest Rohwer, San Diego State University
The Human Virome

Saturday, 18 February

Revealing the Universe

Space missions are often multinational collaborations. The required expertise to pursue them often spans across several nations, and their costs and benefits can be shared globally.

The Fermi Gamma-Ray Mission: Transforming Our View of the High-Energy Universe

Organized by Saeqa Dil Vrtilek, Harvard-Smithsonian Center for Astrophysics; Julie McEnery and Neil Gehrels, NASA Goddard Space Flight Center

SPEAKERS

Julie McEnery, NASA Goddard Space Flight Center
The Fermi Mission and the High-Energy, Gamma-Ray Sky
David Smith, Centre d'Etudes Nucléaires de Bordeaux Gradignan
Pulsars
Peter Meszaros, Pennsylvania State University
Gamma Ray Bursters
Alan Marscher, Boston University
Gamma Ray Blazars: Jets from Super Massive Black Holes in Galactic Nuclei
Luca Latronico, Istituto Nazionale di Fisica Nucleare
The Spectrum of Cosmic Ray Electrons and Positrons

The Life Cycle of Matter in Galaxies: Results from the Herschel Space Observatory

Organized by Margaret Meixner, Space Telescope Science Institute

SPEAKERS

Paul Goldsmith, Jet Propulsion Lab
The Herschel Space Observatory: An International Astronomical Submillimeter Observatory
David A. Neufeld, Johns Hopkins University
The Formation of Interstellar Molecules
Christoffel Waelkens, Instituut voor Sterrenkunde
The Mass Loss Return from Stars to the Interstellar Medium
Peter G. Martin, University of Toronto
Insights on Star Formation in the Milky Way: Cold, Dusty Beginnings
Christine Wilson, McMaster University
Dust, Gas, and Star Formation in Nearby Galaxies: New Results
Asantha R. Cooray, University of California, Irvine
Dust, Gas, and Stars: Astrophysics of Matter in High-Redshift Galaxies

Sunday, 19 February

Climate Change in Northern Latitudes

In the Northern regions, the melting of Arctic ice is just the tip of the iceberg. This frozen region “hides” 1,700 billion tons of organic carbon, the melting of which could accelerate warming.

Causes and Effects of Relative Sea-Level Changes in the Northeast Pacific

Organized by Brian F. Atwater, U.S. Geological Survey (USGS), Seattle; C.K. Shum, Ohio State University; Ester Szein, U.S. National Academies

SPEAKERS

John J. Clague, Simon Fraser University
Impacts of Rising Seas on the British Columbia Coast in the 21st Century
Denise J. Reed, University of New Orleans
Surviving Sea-Level Rise: What Can Be Done To Maintain Viable Coastal Wetlands?
David Flanders, University of British Columbia
Flood Adaptation Near Vancouver: A Regional Adaptation Collaborative

DISCUSSANTS

Margaret Davidson, NOAA; Philip R. Hill, Geological Survey of Canada

Forest Fires in Canada: Impacts of Climate Change and Fire Smoke

Organized by Charmaine Dean, Simon Fraser University

SPEAKERS

Mike Brauer, University of British Columbia
Fires, Smoke, and Health: Impacts and Prevention
Richard Routledge, Simon Fraser University
Fire History in Ponderosa Pine Grasslands: Lessons from the Past
Douglas Woolford, Wilfrid Laurier University
Detecting Temporal Changes in the Seasonality of Forest Fire Risk in Canada
Fay Johnston, University of Tasmania
The Estimated Global Mortality Burden Attributable to Landscape Fire Smoke
Mike Flannigan, Natural Resources Canada
Global Fire and Climate Change
Francis Zwiers, University of Victoria
Has There Been a Human Effect on Surface Air Temperature Extremes Regionally?

Northern Soils: What if They Thaw?

Organized by Luca Montanarella and Geraldine Barry, European Commission, Joint Research Center (JRC)

SPEAKERS

Luca Montanarella, JRC Institute for Environment and Sustainability
What Lies Beneath the Soils of the Northern Circumpolar Region?
Charles Tarnocai, Agriculture and Agri-Food Canada
Soil Organic Carbon Pools in the Northern Circumpolar Region
Charles Koven, Lawrence Berkeley National Laboratory
Frozen Soil Carbon and Its Impact on Climate Change

Special Sessions

Thursday, 16 February

A Global Movement in Support of Inquiry-Based Science Education

International science academies will showcase their efforts to support quality science

education through discussions, poster sessions, and hands-on demonstrations. Academies with a proven history of reaching out to and involving science, technology, education, and mathematics (STEM) professionals can mentor those academies and other science organizations that have little or no experience in this area.

Responsible Professional Practices in a Changing Research Environment

Research integrity is at the core of doing good science, and is a critical factor for achieving accountability. The primary context for the workshop will be international collaborations, with special coverage of: investigator and institutional responsibilities in such research; research integrity issues in cross-cultural research; and strategies for preparing graduate students and post-docs for dealing with ethical issues that can arise in international partnerships.

Symposium Tracks

Climate

Adapting to Climate Change: The Case of Bangladesh

Organized by James Scott Hauger, Asia-Pacific Center for Security Studies; A.N.M. Muniruzzaman, Bangladesh Institute of Peace and Security Studies

Chemistry in the Clouds: Impacts of Aerosols on Climate Change

Organized by Kimberly Prather, Scripps Institution of Oceanography; Vicki Grassian, University of Iowa

Climate Change and Human Evolution: Problems and Prospects

Organized by Mark Collard, Simon Fraser University, Burnaby, BC; Bernard Wood, George Washington University

Climate Change and the Long-Term Sustainability of Human Societies

Organized by Thomas McGovern, City University of New York; Margaret C. Nelson, Arizona State University

Climate Solutions: The Challenges of Getting to 350

Organized by Charles H. Greene, Cornell University

Designing Marine-Protected Area Networks Within Changing Global Climate Conditions

Organized by Robert J. Brock, National Oceanic and Atmospheric Administration (NOAA); Anice Anderson, Private Consultant

Developing Integrated Understanding of Global Climate Using Dynamic Visualizations

Organized by Marcia Linn, University of California, Berkeley

The Future of Ecological Communities Under Climate Change: No Analog?

Organized by David Skelly, Yale University, New Haven, CT; Mark Urban, University of Connecticut; Phoebe Zarnetske, Oregon State University

International Lessons in Cyberspace: Student Learning About Climate Change

Organized by Kirsten H. Sanford, Specialty Studios

Improving Societies Ability To Make Climate Decisions: A Synergetic Model

Organized by Frank Niepold, NOAA

Indigenous Perspectives on Climate Change

Organized by Robert E. Megginson, University of Michigan, Ann Arbor

Non-Carbon Dioxide Greenhouse Gases and Aerosols: Climate Science Information for Decisions

Organized by Venkatachalam Ramaswamy, NOAA Geophysical Fluid Dynamics Lab

Collaboration

Analogy in Applications of Mathematics and Statistics to Other Disciplines

Organized by Benjamin Mann, Ayasdi Inc.; Jack Morava, Johns Hopkins University

Coordinating, Learning, and Sharing Best Practices Among Scientific Diaspora Networks

Organized by Marcelo Vences, National Science Foundation (NSF); Pallavi Phartiyal, AAAS Science and Policy Programs

Documenting a Changing Ocean Through International Multidisciplinary Collaborations

Organized by Roger Francois, University of British Columbia, Vancouver; Roberta Hamme, University of Victoria; Andrey Proshutinsky, Woods Hole Oceanographic Institution

The Earth Microbiome Project: Modeling the Microbial Planet

Organized by Jack A. Gilbert, Argonne National Laboratory

Global Knowledge Collaborations: The Atlas of Islamic World Science and Innovation

Organized by Naser Faruqi, International Development Research Center

Global Knowledge: The Challenge of Diversity and Localism

Organized by Sandra D. Mitchell, University of Pittsburgh

HUBzero: Building Collaboratories for Research on a Global Scale

Organized by Michael McLennan, Purdue University

New Concepts in Integrating Arts and Science Research for a Global Knowledge Society

Organized by Rieko Yajima, AAAS Center for Science, Policy and Society Programs; Gunan Nadarajan, Maryland Institute College of Art

Particle Physics: Pushing Back the Frontiers of Global Collaboration

Organized by Robert McPherson, University of Victoria; William Trischuk, University of Toronto

Physical Sciences Approaches to Cancer: Growing Interdisciplinary Collaborations

Organized by Jan Liphardt and Saheli Datta, University of California, Berkeley

Population, Development, and Environment: Solutions for Global Challenges

Organized by E. Megan Davidson Averill and Tricia Petruney, FHI 360

Quantum Information Science and Technology: A Global Perspective

Organized by Charles W. Clark, Joint Quantum Institute; Amy Wang, Tsinghua University

Successful Interdisciplinary Collaboration: Insights from Practice and Theory

Organized by Melanie Roberts, University of Colorado, Boulder; Edward G. Derrick, AAAS Center for Science, Policy, and Society Programs

Toward a Global Lab: Building Science Capacity in Developing Countries

Organized by Kathryn L. Lovero and Bertram Koelsch, University of California, San Francisco; Lina Nilsson, University of California, Berkeley

Transcending Interdisciplinary Research Barriers: Best Practices for Mobilizing Knowledge

Organized by Dawn R. Bazely, York University, Toronto; Andrew Tanentzap, Biodiversity and Conservation, Landcare Research

Communication

Beyond Climate Models: Rethinking How To Envision the Future with Climate Change

Organized by Arnim Wiek, Arizona State University, Tempe; Olaf Gerhard Schroth and Stephen Sheppard, University of British Columbia

Communicating Climate Change: Meeting the Challenge of Broad Engagement

Organized by Thomas R. Karl, NOAA National Climatic Data Center

Community-Engaged Scholarship: Co-Producing Knowledge for Societal Impact

Organized by Sarena Seifer, University of Guelph

Endangered and Minority Languages Crossing the Digital Divide

Organized by K. David Harrison, Swarthmore College; Claire Bower, Yale University

Everyone's an Expert but Who Is Listening?

Organized by Jan Riise, European Science Events Association

Globalization Then and Now: Scale, Complexity, and Communication of Sustainability

Organized by Carey King, University of Texas, Austin; Joseph Tainter, Utah State University, Logan

Innovations in Reducing International Knowledge Isolation

Organized by Charles Dunlap, CRDF; Vinton Cerf, Google Inc.

Misreporting Fukushima: A Failure of Science Journalism with Global Repercussions?

Organized by Julia Wilson, Sense About Science; Michael Hanlon, *The Daily Mail*

Perspectives from Science Media Centers: Should Scientists Bother with Media?

Organized by Dacia Herbulock, Science Media of New Zealand; Penny Park, Science Media Centre of Canada

The Roles of Citizen Science and Serious Leisure in Public Engagement with Science

Organized by Eric Jones, University of North Carolina, Greensboro; Martin Storksdieck, U.S. National Research Council

Science, Sustainability, and the Arts

Organized by Eugene A. Rosa, Washington State University; Thomas Dietz, Michigan

State University, East Lansing

Sharing Science with the Public Worldwide: Resources for Informal Education Outreach

Organized by Larry Bell, Museum of Science, Boston

Unmuzzling Government Scientists: How To Re-Open the Discourse

Organized by Binh An Vu Van, Association des Communicateurs Scientifiques, Montreal

Using Pop-Culture Icons To Slip Science into the Mainstream

Organized by E. Paul Zehr, University of Victoria

Culture

The American Society in 2035

Organized by John T. Cacioppo, University of Chicago

Archaeoacoustics: Did Ancient Civilizations Use Acoustic Design To Create Powerful Ritual Spaces?

Organized by David Lubman, Acoustical Society of America and Institute of Noise Control Engineering

Constructing a Human World Fit for Nature

Organized by Mimi E. Lam, University of British Columbia, Vancouver

Creating a Global Knowledge Society: Lessons from History, Philosophy, and Sociology

Organized by Heather E. Douglas, University of Tennessee, Knoxville

Culture and Controversy in Science Education Engagement

Organized by Gregory Van Doren, White Earth Tribal and Community College; Lawrence K. Duffy, University of Alaska, Fairbanks

Early Career Scientists in a Flat World

Organized by Rees Kassen, University of Ottawa

The Effects of Early Experience on Lifelong Functioning: Commitment and Resilience

Organized by Stephen J. Suomi, Eunice Kennedy Shriver National Institute of Child Health and Human Development; Janet F. Werker, University of British Columbia, Vancouver

Gesture, Language, and Performance: Aspects of Embodiment

Organized by Philip Rubin, Haskins Laboratories

Globalizing Indigenous Architecture: The Power of Tradition, Providing for the Future

Organized by Omeasoo Butt, University of Saskatchewan

Late Talkers in Any Language: Finding Children at Risk Worldwide

Organized by Nan Bernstein Ratner, University of Maryland, College Park

Making Visible the Invisible

Organized by Lynnette Leidy Sievert, University of Massachusetts, Amherst

Neuroscience and Criminal Justice in the 21st Century: A Cross-Country View

Organized by Michael J. Zigmond, University of Pittsburgh; Judy Illes, University of British Columbia, Vancouver

Scientific Humanists and Humanistic Scientists: Flattening the World with Anthropology

Organized by Agustin Fuentes, University of Notre Dame

Six Things Everyone Cares About: Connecting Ecosystems and Human Well-Being

Organized by Mary Ruckelshaus and Anne Guerry, Natural Capital Project

The Social Consequences of Mass Imprisonment in the United States

Organized by William Alex Pridemore, Indiana University, Bloomington

Development

Anthropology and Engineering: Technological Innovations in Global Health

Organized by Margaret Bentley, University of North Carolina, Chapel Hill; Ram Sriram, National Institute of Standards and Technology (NIST); Don Giddens, Georgia Institute of Technology

Bridging Conservation and Development: A Comparison of Latin America and Africa

Organized by Claudia Romero, University of Florida, Gainesville; Jean-Gael E. Collomb, NSF

Conquering the Final Frontier: The Importance of Space Technologies in All that We Do

Organized by Stephan Lechner, JRC Institute for Protection and Security of the Citizen; William Murtagh, NOAA Space Weather Prediction Center

The Future of Mineral Resource Dependence in the 21st Century

Organized by Samir Doshi, Queen's University, Kingston; Saleem Ali, Institute for Envi-

ronmental Diplomacy and Security

Make It Fit: Supporting a Decent Standard of Living Within Planetary Boundaries

Organized by Stephen Polasky, University of Minnesota, St. Paul

Making Science Work for Development: Case Studies and Best Practices

Organized by Sara E. Farley, Global Knowledge Initiative

Marine Biological Diversity: Who Owns the Greatest Reservoir of Unexplored Diversity?

Organized by Curtis A. Suttle, University of British Columbia, Vancouver; Carlos M. Duarte, Consejo Superior de Investigaciones Científicas and the University of the Balearic Islands

Research as a Portfolio of Projects for Building Our Global Knowledge Society

Organized by Jonathan D. Linton, University of Ottawa; Nick Vonortas, George Washington University

Searching for the Right Space for Innovation

Organized by Peter W.B. Phillips, University of Saskatchewan

Speeding Rates of Innovation

Organized by Jessica Hunt, Gerson Lehrman Group

Discovery

Autophagy: An Emerging Therapeutic Target in Human Disease

Organized by Sharon M. Gorski, BC Cancer Agency and Simon Fraser University; Julian J. Lum, BC Cancer Agency

Biomimetic Chemistry: What Miss Muffet Learned from Spiders, Lizards, and Sea Monsters

Organized by Deborah Leckband, University of Illinois, Urbana-Champaign

Chemical Evolution: From Prebiotic Soup to Intelligent Molecules of the Future

Organized by Cynthia J. Burrows, University of Utah, Salt Lake City; David G. Lynn, Emory University

Excursions into the Mathematics of Medical Imaging

Organized by Jonathan Taylor, Stanford University

Illuminating the Obesity Epidemic with Mathematics

Organized by Carson Chow, U.S. National Institutes of Health (NIH)

Imaging and Controlling Molecular Dynamics with Ultrashort Laser Pulses

Organized by Andre D. Bandrauk, Université de Sherbrooke; Paul B. Corkum, University of Ottawa

Isotopes for Science and Medicine: Rare, Radioactive, and Useful

Organized by Timothy Meyer, TRIUMF; Gene Sprouse, American Physical Society

NanoCellulose: An Abundant, Sustainable, Versatile Biopolymer

Organized by Barbara Illman, U.S. Forest Service

New Frontiers in the Radio Universe: Protoplanetary Disks to the Far Universe

Organized by Mark T. Adams, National Radio Astronomy Observatory (NRAO)

Pulsars: Astronomical Gifts that Keep on Giving

Organized by Mark T. Adams, NRAO, Charlottesville, VA

Quantum Computing: Current Status and Future Prospects

Organized by John Preskill, California Institute of Technology

Quantum Information Technologies: A New Era for Global Communication

Organized by Martin Laforest, Institute for Quantum Computing; Lisa Lambert, Perimeter Institute for Theoretical Physics

Solar Effects on Earth's Atmosphere and Climate: Results from Space Observatories

Organized by Nancy D. Morrison, University of Toledo

Things that Go Bump: The Latest Discoveries in Particle Physics

Organized by Kendra Snyder, Brookhaven National Laboratory; Elizabeth Clements, Fermi National Accelerator Laboratory

Understanding Cellular Machinery Through X-Ray Imaging

Organized by Louis J. Terminello, Pacific Northwest National Laboratory

Education

Demand-Driven Science: Engaging the Future Generation in Solving Tomorrow's Problems

Organized by Mikkel Bohm, Danish Science Communication; Herbert Munder, Science in Dialog

Beyond Evolution: Religious Questions in Science Classrooms

Organized by Peyton West and Jennifer Wiseman, AAAS Dialogue on Science, Ethics

and Religion; Peter Hess, National Center for Science Education

Connecting Education and Research on Retention in Engineering

Organized by Yolanda George, AAAS Education and Human Resources; Elizabeth Litzler and Suzanne Gage Brainard, University of Washington, Seattle

Excellence Gaps in K-12 Education: Race, Gender, and Poverty and High Achievement

Organized by David J. Rutkowski and Jonathan A. Plucker, Center for Evaluation and Education Policy

Interconnected Communities for Biological Research: Integrating Undergraduates

Organized by Susan Singer, Carleton College; M. Patricia Morse, University of Washington Friday Harbor Laboratories

Interdisciplinary Science: Teaching the Key Points of Integration

Organized by David Lawrie and Paul Lu, University of Alberta, Edmonton

Making Better Scientists: Philosophy of Science in Practice

Organized by Hanne Andersen, Aarhus University

Mismatched Resources: Underrepresented STEM Faculty in the Global Knowledge Society

Organized by Julia E. Melkers, Georgia Institute of Technology

More than a Field Trip: Developing Student Leaders To Address Global Challenges

Organized by Julian Marshall and Fred Rose, University of Minnesota, St. Paul

Moving (Actively) from Vision to Change in Undergraduate Biology Education

Organized by Jay B. Labov, U.S. National Academy of Sciences; James Young, Yale University

New Findings in Broadening Participation Research in STEM Education

Organized by Jolene Jesse and Jessie DeAro, NSF

Online 21st Century Science Assessments

Organized by Edys Quellmalz, WestEd

The Role of Standards in Achieving Science Literacy: A Multi-Country Perspective

Organized by Martin Storksdieck, U.S. National Research Council

Teaching Science Through Language

Organized by Anne Lobeck, Western Washington University

Energy

Can a Smarter Grid Slow Down Climate Change While Accelerating Energy Independence?

Organized by Hassan Farhangi, British Columbia Institute of Technology

Can Science and the Public Collaborate on the Global Future of Nuclear Waste?

Organized by Sharon M. Friedman, Lehigh University; Eugene Rosa, Washington State University

Carbon Dioxide Capture and Storage: A Global Solution to a Global Challenge

Organized by Carlos Saraiva Martins, European Commission

The Climate and Health Impacts of Cooking with Biomass

Organized by Hisham Zerriffi and Mike Brauer, University of British Columbia, Vancouver

Energy Storage for Integration of Renewable Energy Sources into the Power Grid

Organized by Igor Shvets, Trinity College Dublin

Food, Feed, and Fuel: Optimizing Economic and Sustainable Biofuel Production

Organized by Nicole Stricker, Idaho National Laboratory; Anice Anderson, Private Consultant

Hydraulic Fracturing of Shale: Building Consensus Out of Controversy

Organized by Raymond Orbach, University of Texas, Austin

Low-Carbon Innovation for an Electricity-Dependent World

Organized by Julie Wright and R.J. Taylor, Waterloo Global Science Initiative

Putting Scientific Breakthroughs to Work in Support of Renewable Energy

Organized by Elizabeth C. Weatherhead and Suzanne Van Drunick, University of Colorado, Boulder

Routes to Enhanced Photosynthesis: Harvesting Sunlight for More Food and Fuel

Organized by Matt T. Goode, Biotechnology and Biological Sciences Research Council

The True Costs of Coal

Organized by Samir Doshi, Queen's University, Kingston; Paul Epstein, Center for Health and the Global Environment

Environment

Acid-Washed Genes and Altered Ecosystems: Biological Tales of Ocean Acidification

Organized by Gretchen Hofmann, University of California, Santa Barbara, CA; Heather M. Galindo, COMPASS

Blue Carbon, Green Opportunities: Innovative Solutions To Protect Coastal Ecosystems

Organized by Ariana Sutton-Grier, NOAA; Stephen Emmett-Mattox, Restore America's Estuaries; Stephen Crooks, Environmental Science Associates and Philip Williams and Associates

The Costs of Conservation: Impacts on Coastal Livelihoods, Health, and Equity

Organized by Joshua E. Cinner, James Cook University; Patrick Christie, University of Washington, Seattle

Historical Biocomplexity in the North Pacific Ocean: Lessons from the Past

Organized by Herbert D.G. Maschner, Idaho Museum of Natural History; Andrew Trites, North Pacific Universities Marine Mammal Research Consortium

Life in the Pressure Cooker: Evolution at the Bottom of the Ocean

Organized by Sven Thatje, University of Southampton

The Magnitude 9.0 Tohoku Earthquake and Tsunami: Significance for Japan and the World

Organized by William U. Savage, University of Nevada, Las Vegas; Susan Bilek and Glenn Spinelli, New Mexico Institute of Mining and Technology

The Ocean Health Index: Diagnosis for a Crowded Blue Planet

Organized by Karen L. McLeod, Communication Partnership for Science and the Sea (COMPASS); Benjamin S. Halpern, University of California, Santa Barbara; Steven Katona, Conservation International

Predicting the Future Ocean: The Nereus Program

Organized by Villy Christensen, University of British Columbia, Vancouver

Responding to and Recovering from Catastrophic Events: The Road to Resilience

Organized by Ann M. Lesperance, Pacific Northwest National Laboratory

Sustaining People and Oceans: Governance in Marine Social-Ecological Systems

Organized by Natalie C. Ban and Vanessa Adams, James Cook University

Swimming in Sick Seas

Organized by Andrew Trites, University of British Columbia, Vancouver; Mike E. Grigg, NIH; Stephen A. Raverty, British Columbia Ministry of Agriculture and Lands

Toward Stabilization of Net Global Carbon Dioxide Levels

Organized by Paul M. Bertsch, University of Kentucky, Lexington, Ester Szein, U.S. National Academies

Vulnerability and Adaptation to Oil Spills

Organized by So-Min Cheong, University of Kansas, Lawrence

Water Security: Multidisciplinary Responses to a Global Challenge

Organized by Karen Bakker, University of British Columbia, Vancouver; Howard Wheeler, University of Saskatchewan; Dennis Lettenmaier, University of Washington, Seattle

Food

Bringing a Global Plant Perspective to Issues that Threaten Humankind

Organized by Melvin J. Oliver, USDA Agricultural Research Service

The Compound Effects of Climate Variability and Climate Change on Food Security

Organized by Rosamond Naylor, Stanford University

Do Public Health and Food Safety Policies and Regulations Impede Innovation?

Organized by Rickey Yada, University of Guelph

Emerging Risks in the Global Food System

Organized by William E. Fry, Cornell University

Enhancing Information Flow for Global Food Security in the Face of Climate Change

Organized by Steven L. Fales and Eugene S. Takle, Iowa State University; Mannava Sivakumar, World Meteorological Organization

Sub-Saharan Africa: Livestock Science Provides New Hope and Faces New Challenges

Organized by Rodney A. Hill, University of Idaho, Moscow; Albert G. Medvitz, McCormick Sheep and Grain

The Next Agricultural Revolution: Emerging Production Methods for Meat Alternatives

Organized by Nicholas J. Genovese, University of Missouri, Columbia; Vladimir Mironov, Medical University of South Carolina

The Role of Information and Communications Technology in Making Sense of Global Food Prices

Organized by Jacques Delince, JRC Institute for Prospective Technological Studies; Geraldine Barry, European Commission JRC

Underreported Yet Overoptimistic: Fisheries Catch Reconstructions and Food Security

Organized by Sarah Harper and Dirk Zeller, University of British Columbia, Vancouver

Who Will Organize and Fund Global Research Efforts in Agriculture Sustainability?

Organized by Catherine Woteki, USDA

Health

Aging: Cells to Society

Organized by John Disterhoft, Northwestern University Medical School

Autism: Genetic, Epigenetic, and Environmental Factors Influencing Neural Networks

Organized by Cindy Lawler, National Institute of Environmental Health Sciences; Isaac Pessah, University of California, Davis

Comprehensive Approaches for HIV/AIDS Prevention and Control

Organized by Yiming Shao, National Center for AIDS/STD Control and Prevention; Myron Cohen, University of North Carolina, Chapel Hill

Curing Spinal Cord Injury: The Need for Global Collaboration

Organized by Michael Fehlings, Toronto Western Hospital; Thomas R. Oxland, International Collaboration on Repair Discoveries

Defeating Alzheimer's Disease To Avoid the Looming Worldwide Health Crisis

Organized by Patrick L. McGeer, University of British Columbia, Vancouver

Fifty Years of the Pill: Risk Reduction and Discovery of Benefits Beyond Contraception

Organized by Kristina D. Chadwick, Bristol-Myers Squibb; Brinda Mahadevan, Abbott Laboratories

Genomics and Cancer: A Global Challenge Needing Global Solutions

Organized by Joseph Connors, BC Cancer Agency

Has the Women's Health Initiative Hormone Therapy Trial Helped Improve Women's Health?

Organized by Gilbert S. Omenn, University of Michigan, Ann Arbor; Andrea LaCroix, Fred Hutchinson Cancer Research Center

The Mouth as a Global Window to Systemic Health

Organized by Carolyn Gibson, University of Pennsylvania School of Dental Medicine, Philadelphia; Clark Spencer Larsen, Ohio State University, Columbus

Norovirus: The Modern Scourge of Food and Family

Organized by Ewen C.D. Todd, Ewen Todd Consulting

The One Health Vision: From Institutional Support to Local Practice

Organized by Barbara Hyde, American Society for Microbiology

Personalized Medicine: Improving Health Outcomes Through Research

Organized by Danièle St-Jean, Canadian Institutes of Health Research

Stem Cell and Cell Therapy Approaches to Understanding Cell Injury and Wound Healing

Organized by Jeffrey J. Yourick, U.S. Food and Drug Administration; John S. Graham, U.S. Army Medical Research Institute of Chemical Defense

Toward the Control of HIV and AIDS Through Comprehensive Treatment as Prevention

Organized by Julio Montaner, BC Centre for Excellence in HIV/AIDS and University of British Columbia, Vancouver; Mark Ouellette, Canadian Institute of Health Research

Winning: Superbugs or Surveillance and Science?

Organized by Patricia L. Keen, University of British Columbia, Vancouver

Information

Accelerating Scientific Progress Through Public Availability of Research Data

Organized by Heather A. Piwowar, NESCent and the University of British Columbia, Vancouver; Michael C. Whitlock, University of British Columbia, Vancouver

Applying Assistive Technology To Improve Quality of Life

Organized by Stephan Lechner, JRC Institute for Protection and Security of the Citizen; Geraldine Barry, European Commission JRC

Architectural Precautions in Networked Networks

Organized by Mariam Thalos, University of Utah, Salt Lake City

Data to Knowledge to Action: Computational Science in a Global Knowledge Society

Organized by Erwin P. Gianchandani, Computing Research Association

Human Rights: Quantitative Methods in the Age of Information and Communication

Organized by Nicholas P. Jewell, University of California, Berkeley

Innovating Innovation: Open Source Successes for Neglected Diseases and Beyond

Organized by Laura W. Musselwhite and Andreas Pilarinos, Universities Allied for Essential Medicines; Mike Gretes, Oregon State University, Corvallis

Is There Life Beyond Moore's Law?

Organized by Sankar Basu and Robert J. Trew, NSF

Leveling the Global Playing Field: Global Inferences from Reliable Global Samples

Organized by Daniel Pauly and Kristin Kleisner, University of British Columbia, Vancouver

Tracking Progress: Success and Failure of Biologging in Protecting the Global Ocean

Organized by Sara M. Maxwell, Marine Conservation Institute and University of California, Santa Cruz; Kristen M. Hart, USGS

Updating the International System of Units: The Foundation for Science and Technology

Organized by Alan G. Steele, National Research Council of Canada; James K. Olthoff, NIST

Web Surveillance: Fighting Terrorism and Infectious Diseases

Organized by Ming-Hsiang Tsou, San Diego State University

Whole-Earth Simulations for Decision-Making: Realistic Goal or Pipe Dream?

Organized by William J. Feiereisen, Intel Corp.

Policy

Assessing Assessments: How Do They Work for Environmental Policy?

Organized by Dale Jamieson and Milena Wazeck, New York University

Can Community Models Help Solve Global Challenges?

Organized by Howard Grimes, Washington State University

Changing Paradigms in the Delivery of Health Care in the United States

Organized by Virginia R. Carson, Chapman University

Declaration of Rights for Cetaceans: Ethical and Policy Implications of Intelligence

Organized by Thomas I. White, Loyola Marymount University, Redondo Beach; Stephanie J. Bird, Science and Engineering Ethics

Exploding Myths on Reactor Security, Harm Reduction, and Genetically Modified Organisms

Organized by Aidan Gilligan, European Commission JRC

Global Challenges to Peer Review of Scientific Publications

Organized by Leonor Sierra and Julia R. Wilson, Sense About Science

The Global Quest for Excellence

Organized by Suzanne Fortier, Natural Sciences and Engineering Research Council of Canada, Ottawa, ON

Harm Reduction: Policy Change To Reduce the Global Toll of Smoking-Related Disease

Organized by Gilbert L. Ross, American Council on Science and Health

International Comparisons of Research and Development Policy

Organized by Patrick J. Clemins, AAAS Office of Government Relations

Redesigning the Governance of Science, Technology, and Innovation After Japan's Earthquake

Organized by Tateo Arimoto and Kazuhito Oyama, Japan Science and Technology Agency; Yuko Harayama, Organization for Economic Cooperation and Development

Science-Informed Public Engagement: Building Support for Policy

Organized by Michael M. Burgess, University of British Columbia, Vancouver

Scientific Integrity Reform in the U.S. Administration: Pitfalls, Progress, and Plans

Organized by Francesca T. Grifo, Union of Concerned Scientists

Whole-Ocean Economics: Global Fisheries Analysis Reveals Potential for Policy Action

Organized by U. Rashid Sumaila, University of British Columbia, Vancouver



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POSITIONS OPEN



C V STARR FOUNDATION FELLOWSHIPS in Neuroscience

The Princeton Neuroscience Institute aims to recruit and support one or two exceptional individuals to fill our C V Starr Fellowships in Neuroscience. These are individuals who are expecting or have recently obtained a Ph.D. degree in neuroscience or areas relevant to neuroscience (molecular biology, psychology, computer science, engineering, physics, or mathematics).

The PNI focuses on interdisciplinary research in neuroscience, spanning from molecular, cellular, and genetic approaches to systems and human cognitive neuroscience. PNI houses state of the art facilities for experimental research in all of these areas as well as advanced computational resources that support its emphasis on theoretical and quantitative approaches to neuroscience. To ensure full consideration, all materials must be received by February 15, 2012.

The C V Starr Fellowship program provides a generous salary and a modest annual research budget. Fellows are encouraged to pursue their own independent research with the guidance and mentorship of a core faculty member in the Institute, although research projects where the Fellow works directly with a core faculty member will also be considered. Fellows also have the opportunity to teach in our Institute programs, subject to sufficient enrollments and approval of the Dean of the Faculty.

For more information about the Institute, see **website:** <http://www.princeton.edu/neuroscience/>.

Applications should be submitted online at **website:** <https://jobs.princeton.edu/> by February 15, 2012. (**Requisition #0110837**). Candidates must submit curriculum vitae, a list of publications, a statement of research interests and goals, and the contact information for three references. Finalists will be invited to Princeton to present a talk concerning their current research.

Princeton University is an Equal Opportunity Employer and complies with applicable Equal Employment Opportunity and Affirmative Action regulations.

ASSISTANT PROFESSOR IN BIOLOGY

The Biology Department at New Mexico Tech seeks a tenure-track Assistant Professor. The successful candidate must be qualified to teach an upper-division course in animal or human physiology, as well as additional undergraduate and graduate courses. The faculty member will establish a research program in any area of biology, and will involve Master's and undergraduate students in their research (with the possibility of directing Ph.D. students in related departments, e.g., Chemistry, Geology, or Materials). Ph.D. in biology or related field required at the time of application. The position will start as early as August 2012. To receive full consideration, application letters along with curriculum vitae, statement of research interests and teaching philosophy, transcripts, and three letters of recommendation should be received by January 31, 2012 to: **Human Resources 801 Leroy Place, Brown Hall Room 118, Box 142, Socorro, NM 87801.**

FACULTY POSITIONS Medical School

The Saint James School of Medicine, an international medical school (**website:** <http://www.sjsm.org>), invites applications from candidates with teaching and/or research experience in any of the basic medical sciences for its Caribbean campuses.

Senior faculty positions are currently available in Pathology and Anatomy. Applicants must be M.D., D.O., and/or Ph.D.

Teaching experience in the U.S. system is desirable but not required. Retired persons are encouraged to apply. Attractive salary and benefits. Submit curriculum vitae electronically to **e-mail:** sjsmcoordinator@sjsm.org or mail to: **HRDS Inc., 1480 Renaissance Drive, Suite 300, Park Ridge, IL 60068.**

POSITIONS OPEN



POSTDOCTORAL RESEARCH POSITION University of Minnesota Novel Approaches to Prevent Murine Graft-Versus-Host Disease (GVHD)

The University of Minnesota, Department of Pediatrics, has an NIH-funded postdoctoral position available in the area of GVHD and tolerance induction. Current studies are directed toward induction and use of regulatory T cells and blockade of T cell activation and migration. Recent relevant references include: *Blood* (epub November 9), 2011; *Blood* 116:5738, 2011; *Blood* 116:466, 2010.

Successful applicants will have an M.D. and/or Ph.D. degree and a track record of accomplishments and expertise in manipulating immune cells. Highly desirable is additional experience in regulating T cell responses to foreign antigens. Excellent facilities are available in the University of Minnesota Cancer Center. Salary will be competitive.

Applicants should send current curriculum vitae, letter of application and the names and e-mail addresses of two to three references electronically to **Dr. Bruce R. Blazar, Professor, e-mail:** blaza001@umn.edu.

The University of Minnesota is committed to the policy that all persons shall have equal access to its programs, facilities, and employment without regard to race, color, creed, religion, national origin, sex, age, marital status, disability, public assistance status, veteran status, or sexual orientation.

TENURE-TRACK ASSISTANT PROFESSOR of Cognitive Psychology

The Department of Psychology, University of California, Riverside, invites applications for a tenure-track Assistant Professor position in Cognitive Psychology to start July 1, 2012. We seek applicants whose research examines the cognitive neuroscience of human learning and memory. The ideal candidate will contribute to our emerging emphasis in experience-dependent change. Applicants should demonstrate a record of research excellence using methodological approaches involving human behavior, cognitive neuroscience, and/or computational modeling. Individuals with interdisciplinary interests are encouraged to apply.

Applicants should be committed to excellence in undergraduate and graduate education and interest in teaching quantitative methods at the graduate level is preferable. The Ph.D. is required and salary is commensurate with education and experience. Review of applications will begin February 15, 2012 and continue until the position is filled. Interested candidates should send hardcopies of their curriculum vitae, a cover letter describing research and teaching interests, reprints if available, and arrange to have three letters of recommendation sent to: **Professor Steve Clark, Chair, Cognitive Search Committee, Department of Psychology University of California-Riverside, 900 University Avenue, Riverside, CA 92521.**

The Riverside campus of the University of California is growing rapidly and has an excellent psychology department with a strong record of success in research, teaching, and extramural funding. For information on the Department of Psychology, see our **website:** <http://www.psych.ucr.edu>. The campus is located about 50 miles east of Los Angeles and less than an hour's drive from the area's mountains, deserts, and beaches. *The University of California, Riverside is an Equal Opportunity/Affirmative Action Employer.*

TENURE-TRACK ASSISTANT PROFESSOR of Biology

The Biology Department of Coe College invites applications for a tenure-track position in human anatomy. An earned Doctorate in biology with an emphasis in anatomy is required; demonstrated success teaching anatomy to undergraduates is desirable. For position details and application process, visit **website:** <http://www.coe.edu/aboutcoe/employment>.

UNIVERSITY OF LOUISVILLE

SCIENTIFIC DIRECTOR

Kosair Children's Hospital Research Institute

Department of Pediatrics, University of Louisville School of Medicine

The Department of Pediatrics at the University of Louisville invites competitive applications to fill the position of Scientific Director of the Kosair Children's Hospital Research Institute (KCHRI). The Institute performs basic and translational research on diseases relevant to pediatric patients. KCHRI faculty research is currently focused on two major areas: (1) neurobiology and (2) the neurologic and cardiovascular complications of obesity and diabetes. Additional pediatric relevant basic science research currently occurs in the Cardiovascular Innovation Institute and the James Graham Brown Cancer Center.

The new KCHRI Director will be expected to expand their extramurally funded research program at the University of Louisville while actively leading the expansion of pediatric-relevant basic science research at the University of Louisville. The successful applicant must have demonstrated effective administrative and leadership experience, a strong record of consistent extramural funding, an excellent publication record, and a track record of successfully mentoring clinician-scientists and basic science investigators. The research focus of the new Director and of subsequently recruited faculty would be determined by the new Director. Candidates with an MD, MD/PhD, or PhD will be considered. A tenure-eligible, endowed Chair and substantial financial support are available for the new Director and the continued growth of the KCHRI. The successful candidate will have a primary academic appointment in the Department of Pediatrics and may also have additional joint appointments in basic science and bioengineering departments to facilitate the training of graduate students and faculty. The Director of the KCHRI reports to the Vice-Chair for Research and to the Chairman of the Department of Pediatrics.

The Academic Programs in the Department of Pediatrics at the University of Louisville cover a broad range of basic science, clinical, and translational research topics supported by over 160 faculty, more than 100 residents and fellows, and highly supportive research and administrative staff. Clinical services are provided at the free-standing Kosair Children's Hospital of Norton Healthcare and at regional sites throughout Kentucky. Our pediatric programs have substantial philanthropic support. We have been highly successful in recruiting talented faculty and residents to the University of Louisville and to our very enjoyable community.

Applications will be reviewed until the position is filled with the intent to have a new Director in place by the third quarter of 2012. Please submit a cover letter including a statement of research interests, curriculum vitae, and contact information for three references electronically to **Bradley B. Keller, MD, brad.keller@louisville.edu**, Vice Chair for Research, Department of Pediatrics and Kosair Charities Chair and Chief, Division of Pediatric Heart Research, Cardiovascular Innovation Institute, University of Louisville.

The University of Louisville is an Affirmative Action, Equal Opportunity, Americans with Disabilities Employer, committed to diversity, and in that spirit, seeks applications from a broad variety of candidates.



The Gene and Linda Voiland School of
Chemical Engineering and Bioengineering

Faculty Position in Bioengineering at Washington State University

The Gene and Linda Voiland School of Chemical Engineering and Bioengineering (ChEBE) at Washington State University invites applications for a tenure-track faculty position in bioengineering at the Assistant Professor level. The position will be based at the Pullman campus. Successful candidates must hold a doctorate in bioengineering, chemical engineering, or a closely related discipline. Applications are especially encouraged from individuals with a strong record of publication in high impact, peer-reviewed and archival journals and evidence for potential to establish an externally funded research program. Applicants with research in an area closely related to our current strengths in cellular and protein engineering, cellular/tissue biomechanics, biofilm engineering, biochemical engineering and engineering education are encouraged.

The Voiland School currently has 300 undergraduate students, 60 graduate students, of whom 56 are PhD students, and 18 faculty. During fiscal year 2011, faculty in the school were awarded extramural funds totaling \$4.14 million from industry, NSF, DOE, DOD, NIH, and ONR and created two new companies from technologies developed as a part of their research. During the next year, we plan to add two additional faculty, including the individual who will be hired into this position. Faculty in the Voiland School are conducting interdisciplinary research and have strong interactions with College of Veterinary Medicine, WSU's newly established School for Global Animal Health, and Pacific Northwest National Laboratory, as well as other departments in the university. A successful candidate for this position is expected to establish and maintain a funded, innovative and nationally recognized research program, collaborate with others within the School, the University, or other organizations, and develop and teach graduate and undergraduate core and elective courses.

Interested candidates should submit a letter of application, a detailed curriculum vitae, names and contact information of four references, a statement of research plans and a statement of teaching interests to: www.wsujobs.com/applicants/Central?quickFind=56748. The letter should be addressed to **Dr. Wen-Ji Dong, Chair Search Committee, Gene and Linda Voiland School of Chemical Engineering and Bioengineering, Washington State University**.

Screening of application materials will begin immediately and will continue until the position is filled. Preferred starting date of this new position is August 15, 2012.

*Washington State University is an EEO/AA/ADA Educator and Employer.
WSU employs only U.S. citizens and lawfully authorized non-U.S. Citizens.*



SCHOOL OF MEDICINE
CASE WESTERN RESERVE
UNIVERSITY

VIROLOGY, CANCER VIROLOGY ASSOCIATE/FULL PROFESSOR POSITIONS

Department of Molecular Biology and Microbiology

The Department of Molecular Biology and Microbiology at CWRU School of Medicine is continuing its expansion under our Chair, Dr. Jonathan Karn. We are currently seeking applications for **Associate/Full Professor** level positions. We encourage applications from highly qualified individuals with demonstrated experience in the areas of:

- **Molecular Virology:** Fundamental aspects of Virology (e.g. molecular mechanisms in viral replication; virus assembly and maturation; control of latency; host cell interactions; virus entry mechanisms; viral diversity and immune evasion).
- **Cancer Virology:** Basic virology leading to a fundamental understanding of the molecular basis for tumorigenesis. This includes interests in the discovery of new human pathogens, viral etiology of cancer, viral models for cancer.

Successful candidates will establish a vigorous research program, participate in teaching activities, and interact productively with a nationally-ranked team of basic and clinical scientists interested in the overall areas of HIV/AIDS and host-pathogen interactions, microbiology and infectious diseases.

We stress excellence in research and in teaching.

- Candidates at the Full Professor level should have a Ph.D., an outstanding record of scholarly achievement and record of leadership and service at the national or international level in the profession, and a record of sustained Federal funding.
- Candidates at the Associate Professor level should have a Ph.D., a substantial publication record and an established reputation for professional excellence, and a record of sustained Federal funding.

In addition to newly refurbished laboratory space and a generous start-up package, we offer a highly interactive environment with exceptional intellectual, infrastructural, and administrative support. All candidates should have a Ph.D. and relevant post-doctoral experience, and a record of funding, active research program and a national reputation. Rank and salary will be commensurate with experience.

Please submit an online application including a letter of application, curriculum vitae, brief statement of research goals and accomplishments, and 3 references as PDF files to: **Jonathan Karn, Ph.D., Chair; Virology Search Committee** at: <http://sunshine.case.edu/mvsearch/index.htm>.

In employment, as in education, Case Western Reserve University is committed to Equal Opportunity and Diversity. Women, veterans, members of underrepresented minority groups, and individuals with disabilities are encouraged to apply. Case Western Reserve University provides reasonable accommodations to applicants with disabilities. Applicants requiring a reasonable accommodation for any part of the application and hiring process should contact the Office of Inclusion, Diversity and Equal Opportunity at 216-368-8877 to request a reasonable accommodation. Determinations as to granting reasonable accommodations for any applicant will be made on a case-by-case basis.

Director, Office for Policy in Clinical Research Operations, Division of AIDS

The National Institute of Allergy and Infectious Diseases (NIAID) is seeking an exceptional and visionary leader to take on the role of the director, Office for Policy in Clinical Research Operations (OPCRO) in the Division of AIDS (DAIDS). The OPCRO director is responsible for oversight of a number of critical functions to ensure 1) the sound conduct of clinical trials; 2) compliance with applicable regulations, standards, and good practice guidelines; 3) study participant safety and welfare, and 4) study quality and integrity. The OPCRO director oversees four branches composed of 28 federal employees as well as a number of on-site contractors. The OPCRO director also serves as a key advisor to the DAIDS director and to scientific programs on all clinical research oversight issues; provides objective, experience-based guidance and oversight; and is responsible for identifying and resolving a variety of complex clinical trials management, policy, and administrative issues. These issues require close coordination and interfacing with other programs within NIAID, NIH, other federal agencies, pharmaceutical companies, and patient advocacy and community groups.

Qualifications: Applicants must possess an M.D. or equivalent degree with specialization in internal medicine, infectious diseases, or any other medical specialty that will lead to expertise in infectious diseases research with a focus on HIV/AIDS. In addition, the candidate must have demonstrated skills in 1) working both independently and collaboratively in planning, organizing, and conducting/overseeing clinical trials; 2) serving effectively in clinical research program administration; and 3) effective communications and collaborations.

Application Process: Provide curriculum vitae, bibliography, and a three-page summary explaining 1) your vision for HIV/AIDS clinical research; 2) your reasons for being interested in the position; and 3) the specific leadership skills and experience you would bring to the HIV/AIDS clinical research programs at NIAID. Submit application package to Mr. Robert Gulakowski, Office of the Director, DAIDS, NIAID, 6700-B Rockledge Drive, Room 4140, Bethesda, MD 20892-7620, and reference announcement number DAIDS-11-03.

The deadline for receipt of applications is January 31, 2012. Direct any inquiries to Mr. Gulakowski at rgulakow@niaid.nih.gov or 301-496-0545. All information provided by applicants will remain confidential and will be reviewed only by authorized NIAID officials.



NIAID

National Institute of Allergy and Infectious Diseases

The successful candidate will be appointed under the Title 42(f) authority at a salary commensurate with experience. The maximum annual base salary is \$230,000, with a maximum total annual compensation limit of \$240,000. A full package of benefits is also available, including retirement; health, life, and long-term care insurance; annual and sick leave; and a thrift savings plan (401K equivalent). This position is subject to financial disclosure requirements.

To learn more about NIAID and how you can play a role in this exciting and dynamic research organization, visit us on the Web at www.niaid.nih.gov/careers/dict.



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health



National Institute of Allergy and Infectious Diseases
Proud to be Equal Opportunity Employer

TRANSLATIONAL BIOMEDICAL RESEARCH OPPORTUNITY

Genomic Medicine/Bioinformatics

Sigfried and Janet Weis Center for Research is seeking outstanding independent scientists for full-time research positions at ranks equivalent to Assistant, Associate or Full Professor in the areas of Genomics and Bioinformatics. The Weis Center is a basic and translational research facility of Geisinger Clinic located at Geisinger Medical Center (GMC) in Danville, PA. **Genomic Medicine** is a strategic focus for translational research at Geisinger.

Genomic Medicine is a strategic focus for translational research at Geisinger.

About the position:

- Expertise in laboratory, computational, or statistical genetic approaches
- Expand ongoing research on the genetic basis of disease
- Proven records of innovative research with relevance to human disease
- Collegial environment with collaborative research opportunities

Geisinger Health System's advanced electronic medical record system and health information technology infrastructure allows for electronic capture of clinical data and large biorepository of patient specimens.

Technical resources include instrumentation for confocal, TIRF, and single cell fluorescence imaging, microarray analysis, genotyping, DNA sequencing, and flow cytometry, and an AAALAC-accredited animal facility. Substantial resources are available for start-up, ongoing research support and salary.

Qualified individuals should submit curriculum vitae, statement of research interests and three reference letters to Ms. Kristin Gaul, Weis Center for Research, Geisinger Clinic, via email (kgaul@geisinger.edu). Please refer to position WCR-3638 in the subject line. Applications will be accepted until the positions are filled.

For more information on research programs at Geisinger visit our website at <http://www.geisinger.org/professionals/research/wcr>.

Geisinger Health System is an Affirmative Action/Equal Opportunity Employer

**GEISINGER
HEALTH SYSTEM**

REDEFINING THE BOUNDARIES OF MEDICINE

Chair • Physiology and Biophysics

Stony Brook University School of Medicine (SOM) is seeking candidates and nominees for Chair of the Department of Physiology and Biophysics. The SOM is dedicated to excellence in teaching, research, and clinical care and has a long-standing record of accomplishment in the basic sciences which it seeks to grow as detailed in our strategic plan located at http://www.stonybrookmedicalcenter.org/system/files/strategicPlan_REV_LR.pdf. Candidates should demonstrate the highest level of ethical conduct, a commitment to diversity; possess a track record of recruiting and retaining outstanding educational and research (basic and translational) faculty. Applicants should also possess strong interpersonal and communication skills, a demonstrated ability to provide leadership within a complex organization and strong academic credentials evidenced by a track record of successful extramural funding and scholarly accomplishments. He/she should have a training track record that documents a commitment to medical education and mentoring of young faculty, experience with the management of an academic department within a medical school and in the oversight of education and research budgets; and possess the ability to establish effective operational practices that create an environment that supports the missions of the department.

Required: Ph.D. degree or equivalent. Demonstrated experience in budgetary and personnel management. Must be eligible for a senior faculty appointment, the candidate must meet the criteria established by the School of Medicine (School of Medicine's Criteria for Appointment, Promotion, and Tenure). Please visit our website at: <http://pnb.informatics.stonybrook.edu/> to obtain additional information about the Department of Physiology and Biophysics.

For a full position description and/or application procedures, visit www.stonybrook.edu/jobs (Job Reference #: F-7079-11-12). Applications will be accepted until the position is filled. Nominations and applications including a State employment application and curriculum vitae should be submitted to: William L. Jungers, Ph.D., Distinguished Teaching Professor and Chair, Search Committee Chair, Department of Anatomical Sciences, Health Sciences Center, Tower A, 8th Floor, Room 020,

Stony Brook University,
Stony Brook, NY 11794-8081
Fax #: (631) 444-3947

Stony Brook University/SUNY is an equal opportunity, affirmative action employer.

**STONY
BROOK**
STATE UNIVERSITY OF NEW YORK



FACULTY POSITION MICROBIAL PATHOGENESIS DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY

The Department of Microbiology and Immunology at Dartmouth Medical School seeks to recruit an outstanding candidate who studies aspects of bacterial or fungal pathogenesis or other microbe-host interactions that occur in the context of the lung, the gut, or other mucosal sites. Dartmouth has a highly interactive and stimulating research environment. Thus, individuals with cross-disciplinary interests who combine the study of host-associated microbes or microbial communities with state-of-the-art genetics (e.g. ncRNA), genomics, biochemistry, imaging, and/or bioinformatics are of particular interest. There are also opportunities for interactions with the clinical departments at Dartmouth-Hitchcock Medical Center. The successful applicant will hold a PhD, MD, or MD/PhD degree, and the intent is to recruit a tenure track faculty member at the Assistant or Associate level, however candidates at all ranks are welcome to apply and would be considered for an appointment at a rank commensurate with experience. We seek individuals who will develop or expand upon a robust, independent, extramurally funded research program, and a demonstrated ability to attract external funding is desirable. Recruited faculty will interact closely with members of the Molecular Pathogenesis Program (www.m2p2.org), and the position includes membership in the Molecular and Cellular Biology graduate program (www.dartmouth.edu/~mcb). For further information on the Department of Microbiology and Immunology, see dms.dartmouth.edu/microbio/.

Applications should consist of a curriculum vitae and a brief statement of research interests and future plans, and the candidate should have three letters of reference sent on their behalf. Direct applications to: **Karen Thompson at MIsearch@dartmouth.edu, Department of Microbiology and Immunology, Dartmouth Medical School, Vail Building HB7550, Hanover, NH 03755.** For informal inquiries, contact **Deborah Hogan, dhogan@dartmouth.edu**, or **Bruce Stanton, bruce.a.stanton@dartmouth.edu**. Review of applications will begin immediately and continue until the position is filled.

Dartmouth Medical School is an Affirmative Action/Equal Opportunity Employer and encourages applications from women and members of minority groups.



CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS
Office Block: 2nd floor, Block No. 7, Gruhakalpa Complex,
CDFD M J Road, Nampally, Hyderabad - 500 001
Ph: +91-40-2474 9451 Fax: +91-40-2474 9490, www.cdfd.org.in

SCIENTIFIC GROUP LEADERS (FACULTY) RECRUITMENTS ADVERTISEMENT NO. 1/2012

The CDFD is an autonomous institute supported by the Department of Biotechnology, Government of India, that uniquely blends a high-quality service facility in DNA profiling and diagnostics with basic research in the frontier areas of modern biology. The current research themes, initiated and conducted by its Faculty investigators, include computational and structural biology, genetics and epigenetics, silkworm genomics, molecular pathogenesis, cancer and stem cell biology, cell signalling, and molecular and cellular biology. The Centre also hosts an active and vibrant Ph.D. scholars program in these areas. The Centre invites applications from Indian citizens for SCIENTIFIC GROUP LEADER (FACULTY) positions in any of the areas of modern biology, for which a number of vacancies exist. For all these positions, the candidates will be expected to lead independent groups and to attract extramural research funding.

Applicants should possess a Ph.D. in any branch of Natural Sciences or an M.D., and must have an outstanding track record of scientific productivity, as evident from publications in scientific journals of high impact and/or international patents. Although post-doctoral experience is desirable, exceptional candidates with a fresh Ph.D. may also be considered. The level of appointment, at either Assistant or Associate Professor equivalent, will depend on the length of experience and the quality of scientific productivity.

For further details on the advertised positions and on the ongoing research and service activities, prospective applicants may visit the CDFD website (<http://www.cdfd.org.in>), where the details of application procedure are provided.

There is no last date for receipt of applications, which will be evaluated periodically until all the positions are filled. Shortlisted applicants will be required either to appear before a Selection Committee or to visit the CDFD for a seminar and discussions before a decision is made.



宁波大学
NINGBO UNIVERSITY

Ningbo University invites you to apply for faculty positions

About Ningbo University

Founded in 1986 by Sir Yue-Kong Pao, Ningbo University (NBU) is a young and dynamic university located in the beautiful city of Ningbo by East China Sea, with five campuses covering 160 hectares of land. As a leading comprehensive university in Zhejiang Province, NBU offers programs in economics, law, education, liberal arts, history, science, engineering, agriculture, medicine, and management. The university now receives public funding, as well as continuous support and generous donations from many overseas Chinese and their families including Sir Yue-Kong Pao, Sir Run-Run Shaw, Chao An Chung, Hans Tang, Yue-shu Pao, Cao Guangbiao, Li Dashan, Zhu Xiushan, etc.

Academic Excellence

NBU consists of 19 schools and offers 71 undergraduate programs, 155 master programs, and 7 Ph.D. programs. It enrolls 29,520 students including 25,574 full-time undergraduates and 3,951 graduate students. Currently NBU has 1,330 full-time faculty members and 1,070 administrative staff members. Among them there are 4 academicians, 255 full professors, and 720 associate professors.

Research Achievements

With 77 research institutes and key laboratories, NBU is the center of varieties of research and teaching activities. The research and development initiatives of the university, especially in marine science, information science and technology, engineering mechanics, and material science have contributed greatly to the economic development of the region and have been recognized by numerous national awards. The university library has a CNKI Network Administrative Service Center, and a collection of approximately 3,400,000 books and digital resources.

International Programs

NBU maintains close links to 47 well-known institutions of higher education in Canada, Germany, France, Great Britain, USA, Sweden, Japan, South Korea and Australia. For example, the Sino-Canada joint-educational program is welcomed by international students with 100% satisfaction.

QUALIFICATIONS

Candidates should at least have (a) a Ph.D. degree in a related discipline, (b) adequate teaching ability and a strong passion for teaching, (c) an outstanding research background and influential publication record in recent three years, and (d) an ability to conduct high-quality research and attract external funding.

REMUNERATION & CONDITIONS OF SERVICE

Salary offered will be commensurate with qualifications and experience. Remuneration package will be highly competitive. For applicants with titles of professor or associate professor, salary and housing compensation can be negotiated on the individual basis. For newly graduate PhD and Postdoctoral, initial appointment will be made on a fixed-term contract, with a housing compensation of 600,000 RMB upon fulfillment of the contract requirements. Re-engagement thereafter is subject to mutual agreement.

APPLICATION

Please submit completed application form, CV and cover letter via email to rsc@nbu.edu.cn; by fax at +86 574 8760 0288; or by mail to 31# postbox, Ningbo University, 818 Fenghua Road, Ningbo, China. Application forms can be downloaded from <http://www.nbu.edu.cn/shizi> Recruitment stays open until positions are filled unless otherwise specified. Please visit <http://rsc.nbu.edu.cn> for more details.

INFORMATION ON POSITIONS

SCHOOL OF MARINE SCIENCE

Professor/Associate Professor
Marine Biotechnology/ Marine Sciences/Medical Chemistry/ Ocean Engineering/Marine planning and remote sensing /Sea port and environmental ecology/Marine Geographic Information Science

SCHOOL OF INFORMATION SCIENCE AND ENGINEERING

Professor/Associate Professor/PhD Supervisor
Mobile Communications/Optical Communications/Microwave Communication/ Satellite Communication/Broadband Wireless Communication/The Internet of Things Technology/Graph and Image processing/Audio Signal Processing/Video Signal Processing/Multimedia Signal Processing and Communication/Theoretical Information Processing/Trusted Computing/Software and Theory/Software System Architecture/ Information Security/Circuit and System/Integrated Circuit Design/Mechanical and Electrical Integration

SCHOOL OF SCIENCE

Professor/Associate Professor/Assistant Professor
Condensed Matter Physics/Microelectronics/Optoelectronics/Solar Cell Department of Mathematics/Computational Mathematics/Probability and Statistics / Financial Mathematics

SCHOOL OF MECHANICAL ENGINEERING AND MECHANICS

Professor/Associate Professor
Fluid Mechanics/Vehicle Engineering/Mechanical Engineering/Industrial Design

INTERNATIONAL SCHOOL

Professor/Assistant Professor
Accounting

SCHOOL OF MATERIALS SCIENCE AND CHEMICAL ENGINEERING

Assistant/Associate/Full Professors
Polymer Science/Chemical Engineering/Materials Science

SCHOOL OF LAW

Professor/Associate Professor/Assistant Professor
Criminal Law/Criminal Procedure Law/Civil Procedure Law/Civil Law/Electronic Commerce Law

SCHOOL OF TEACHER EDUCATION

Professor/Associate Professor/Assistant Professor
Curriculum and Teaching Methodology/Higher Education/Preschool Education/Educational Economy and Management/Cognitive Psychology/Educational Psychology/Clinical Psychology/School Psychology/Experimental Psychology/Personality Psychology/Social Psychology/Management Psychology/Computer Graphics and Digital Image Processing/Electronic Music, Game Development/3D Animation and Game Development

SCHOOL OF MARITIME AND TRANSPORTATION

Professor/Associate Professor/Assistant Professor
Department of Logistics and Transportation/Department of Maritime Technology/Department of Marine Engineering/Department of Naval Architecture and Ocean Engineering

SCHOOL OF SPORT SCIENCE

Assistant Professor
Sport Management/Sport Sociology/Human Movement/Sport Training/Sport Physiology

MEDICAL SCHOOL

Professor / Associate Professor
Molecular Biology/Diabetes/Neurobiology/Pathology / Pathogenic Microbiology/ Epidemiology

SCHOOL OF ARTS

Professor / Associate Professor / Assistant Professor
Performance of all areas, Musicology (Ethnomusicology), music composition
Art design of all areas, digital arts, fine art, art history, art/music industry, art/music therapy

SCHOOL OF ARCHITECTURE, CIVIL ENGINEERING

Professor / Associate Professor / Assistant Professor
Architecture Design/Urban Design/ Urban Planning/Architecture Technology/Human Geography/ Physical Geography/Cartography and Geographic Information System/Environmental Technology/Applied Environmental Microbiology



TEXAS TECH UNIVERSITY
Health Sciences Center
Paul L. Foster School of Medicine

Assistant or Associate Professors Center of Excellence in Diabetes and Obesity Research

The Department of Biomedical Sciences and the Center of Excellence in Diabetes and Obesity at the Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center, El Paso, Texas are seeking candidates for tenure track faculty positions at the Assistant or Associate Professor level. This is part of a state-funded initiative to enhance research in diabetes and obesity in the US / Mexico border region. The Center is primarily interested in investigators with research interests in diabetes and obesity, preferably in Hispanic populations or diseases of special interest to Hispanic populations.

Successful candidates are expected to maintain independently funded research programs in obesity and diabetes or a related field. The positions will report to the Chair of the Department of Biomedical Sciences until such time as a Director of the Center of Excellence is identified.

Minimum Qualifications: M.D./D.O. with board certification and eligibility for Texas medical license or Ph.D. in a field related to diabetes/obesity research from an accredited institution of higher education. Minimum 3 years of postdoctoral experience, and a strong publication record.

Preferred Qualifications: Candidates with experience in diabetes/obesity research and experience in using the latest technology preferred. Funded grant support in an area of basic, clinical, or translational research strongly preferred. Regional or emerging reputation in the area of diabetes and obesity research for consideration at the rank of Associate Professor. Interest in assisting with the development of a new Graduate School of Biomedical Sciences strongly preferred. Access to graduate students will depend on development of a successful training program, and we look to the candidates to participate in the development of the program. A willingness to start something new, and to help a new institution to grow and develop, is also a strongly desired characteristic. Good communication skills and demonstrated ability to work in a collegial environment are essential.

The Center will provide start up funds and partial salary support. 50% of salary should be supported by grant funding or a combination of grant funding and clinical/teaching work. Interested candidates must apply online at <http://jobs.texastech.edu>, requisition 84742. A curriculum vitae, a short write up of research interests and three references may be attached and sent to: **Charles Miller, PhD. charles.miller@ttuhsc.edu, Chair, Department of Biomedical Sciences.** The position is open until filled. Application review will begin immediately.

Texas Tech University Health Sciences Center is an Equal Opportunity/Affirmative Action Employer.



Assistant Professor
of Microbiology

Pocatello, Idaho

The Department of Biological Sciences at Idaho State University (www.isu.edu/departments/bios) invites applicants for 9 month, tenure-track position in Microbiology at the Assistant Professor level to start Fall 2012. All areas of Microbiology will be considered. Candidates should outline how they will complement current departmental strengths in microbial molecular genetics and biochemistry, extremophile microbiology, and biomedical research. The successful candidate is expected to develop and maintain a vigorous, externally funded research program, to teach effectively at both the undergraduate and graduate levels and perform professional and university service. A doctoral degree in Microbiology or a related discipline and post-doctoral experience are required. Review of applications will begin January 16, 2012, and will continue until the position is filled. For full consideration, please apply through the Idaho State University Human Resources website (www.isujobs.net).



ISU is an equal opportunity/affirmative action employer. We have an institution-wide commitment to inclusion and diversity and encourage all qualified individuals to apply. Veterans' preference. Upon request, reasonable accommodations in the application process will be provided to individuals with disabilities.

Max Planck Institute for Ornithology



The Max Planck Institute for Ornithology, Vogelwarte Radolfzell, is an internationally renowned research institution working in the field of Eco-Immunology and Migration.

The Max Planck Institute for Ornithology and the University of Konstanz, Chair of Ornithology is seeking, to build up an Interdisciplinary Research Group in Global Computational Ecology,

2 post-doctoral positions in full-time in the areas of movement ecology and remote sensing.

We look for excellent colleagues working in computational ecology / movement ecology on a global scale with emphasis on cross-taxa comparisons of patterns of movement and migratory behavior. The successful candidate may take advantage of the large movement data across various taxa collected at different times with different methods currently stored in the global data base movebank.org. Remote sensing skills would be highly useful. The suggested project centers around the development of algorithms and statistical procedures to tackle efficiently the comparative aspect of movement and also is concerned with the processes in acquisition of heterogeneous animal movement data. Together with the remote sensing experts (particularly the second postdoctoral position) the project should also investigate the interaction of different species and their movement patterns in different environmental contexts. The positions will strengthen the nascent computational ecology group at the University of Konstanz and the Max Planck Institute for Ornithology and the project will be part its ongoing efforts to develop tools and methods to understand animal movement from an ultimate and proximate perspective. A fluent programming in MatLab, SAS, R, C++, Java or any equivalent language is required.

The successful candidates will be excellent, highly motivated and productive postdoctoral scientists. Successful applicants will have demonstrated the ability to perform top international research, ideally using integrative approaches to study broad-scale ecological questions.

The positions are available for 2 years and will be hosted by group leader Dr. Kamran Safi. The researcher can expect an outstanding scientific environment and excellent support at this newly re-established department of the Max Planck Institute for Ornithology in Radolfzell, Baden-Württemberg, at Lake Constance. Our department has close ties with the nearby University of Constance, a 'cluster of excellence' university. The language at the institute is English.

In an effort to employ more people with disabilities, the Max Planck Society specifically encourages people with disabilities to apply for the position. To increase the employment of women in areas where they are under-represented, the Max Planck Society also encourages women to apply for this position. Payment and benefits are according to the German TVöD, with the precise salary level (up to maximum level 14), depending on personal qualifications. There will be further social benefits according to the provision of the public sector.

The position will ideally begin in spring/summer 2012. Screening of applications will begin on January 20, 2012.

For further details, please contact Dr. Kamran Safi (ksafi@orn.mpg.de, phone: +49 (0)7732-150-173).

Applications should be submitted to include your CV, a 2-page visionary research statement, and a list of three references to:

Max Planck Institute for Ornithology
Personalabteilung
Eberhard-Gwinnerstr.
D-82319 Seewiesen
or by email: personal@orn.mpg.de



MAX-PLANCK-GESELLSCHAFT

CAREER TRENDS

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The Baylor Institute for Immunology Research (BIIR) in Dallas, Texas has a mission to translate discoveries in immunology from the laboratory into patient care. Established in 1996, BIIR currently has over 140 members and includes advanced core facilities such as genomics, flow cytometry, confocal microscopy, hybridoma, humanized mice, a BSL-3+ lab and a cGMP laboratory. BIIR is a world leader in translational immunology research, including the areas of dendritic-cell-based cancer vaccines, autoimmunity and infectious diseases. BIIR has over \$16 million funding annually from NIH, CPRIT, the French research agency (INSERM/ANRS), industry and private foundations. BIIR also has very strong support from the Baylor Health Care System.

Baylor recently recruited Yong-Jun Liu, MD, PhD, as the BIIR Director as well as the Vice President and Chief Scientific Officer for Baylor Research Institute. Under this new leadership and direction, BIIR is expanding several of its research programs.

We are recruiting Faculty at all levels for the following areas:

- **Autoimmunity & Inflammation**
- **Cancer Immunology**
- **Genomics & Bioinformatics (Center Director Position also available)**
- **Molecular Immunology**
- **Transplant Immunology (Center Director Position also available)**

We also have **Project Manager** positions available in our autoimmunity and vaccine programs. These positions will be responsible for managing our programs from preclinical/early clinical trial phase through the clinical trial pipeline, including biomarker and vaccine development. Project Managers will work with investigators, regulatory organizations (including the FDA), and other internal and external stakeholders.

For more information on BIIR, please visit our website: www.biiir.org

Apply online at www.baylorhealthcareers.com/science or contact James Smyda at James.Smyda@baylorhealth.edu or 972-291-4573.



Faculty Position in Infectious Diseases Dynamics Modeling

The Vaccine and Infectious Disease Division of the Fred Hutchinson Cancer Research Center seeks applicants for a full-time faculty position at the Assistant or Associate Member level. The position carries a joint or adjunct appointment with the University of Washington at the Assistant or Associate Professor level. The successful candidate's research will focus on mathematical, statistical, and computational methods for understanding the transmission dynamics of human pathogens and how interventions impact those dynamics and/or dynamics of the interaction between host responses and human pathogens. The candidate must hold a doctoral level degree in a quantitative science or in one of the following areas: evolutionary biology, evolutionary ecology, mathematics and biology, population biology, immunology or medicine. The applicant must have a strong track record in the use of deterministic and stochastic modeling, analytic and computational approaches, and use of statistical analysis to understand the interhost or intrahost dynamics and biology of infectious diseases. The candidate must have expertise in areas relating to the pathogenesis of herpes viruses, HIV, influenza, tuberculosis, malaria, dengue and cholera, or other important infectious diseases in humans. This individual will be expected to develop an independent research program. A vital role for this position is to forge collaborations with colleagues at the Hutchinson Center and at the University of Washington; the successful candidate will have a proven ability to establish productive collaborations with theorists, experimental immunologists and infectious diseases researchers. Salary DOE + excellent benefits.

To assure consideration, candidates should apply by **February 25, 2012** by sending a CV, a concise research plan, and the names and contact information for three (3) references to: **Christina Wee, viddfao@fhcrc.org**. Later applications may also be considered if the position is not yet filled.

The University of Washington and the Fred Hutchinson Cancer Research Center are Affirmative Action, Equal Opportunity Employers. The University of Washington and FHCRC faculty engage in teaching, research and service. We are dedicated to the goal of building a culturally diverse and pluralistic faculty and staff committed to teaching and working in a multicultural environment and strongly encourage applications from women, minorities, individuals with disabilities and covered veterans. The University of Washington, a recipient of the 2006 Alfred P. Sloan award for Faculty Career Flexibility, is committed to supporting the work-life balance of its faculty.

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AAAS is here – preparing minority students for careers in science.

Part of AAAS's mission is to strengthen and diversify the scientific work force. To help achieve this goal, AAAS partners with NSF to present the Historically Black Colleges and Universities Undergraduate Program, a conference where students from HBCUs get experience presenting their research, networking with peers, meeting with representatives from graduate schools, and learning about career opportunities. As a AAAS member, your dues support these efforts. If you're not yet a member, join us. Together we can make a difference.

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Universität Hamburg
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HPI
Heinrich Pette Institut
Leibniz-Institut für Experimentelle Virologie



Universität Hamburg Eppendorf



The **Center for Structural and Systems Biology (CSSB)** will be established on campus of the German Electron Synchrotron (DESY) in Hamburg. CSSB is designed to attract outstanding scientists from a variety of regional and national research institutions to establish laboratories and take advantage of the extraordinary technical facilities and methodological opportunities provided by DESY for pioneering work on structural biology. For example, the synchrotron storage ring Petra III and the European X-ray free electron laser XFEL (operational in 2014) will enable scientists to follow over time the dynamics of complex cellular processes at atomic resolution. In particular, CSSB's research focus lies in infection research. Both its concept and infrastructure will make the CSSB a highly innovative alliance institute, enabling it to rapidly gain visibility at national and international levels and develop into a leading research center in the field.

The University of Hamburg together with the University Medical Center Hamburg-Eppendorf (UKE), the Heinrich Pette Institute – Leibniz Institute for Experimental Virology (HPI) and the Bernhard Nocht Institute for Tropical Medicine (BNI) will appoint a total of three professors to establish laboratories at CSSB. Scientists at these institutions study biologically and medically relevant proteins in multi-component complexes which will be investigated by applying biophysical methods at CSSB.

Applicants are expected to demonstrate research experience on an international level as well as a successful track record in acquiring external project funding and carrying out externally funded projects. The University and the University Medical Center Hamburg-Eppendorf places particular value on the quality of teaching. We therefore request applicants to provide details of both their teaching experience and their future teaching objectives.

The University and the University Medical Center Hamburg-Eppendorf aims to increase the number of women in research and teaching and explicitly encourages women to apply. Equally qualified female applicants will receive preference in accordance with the Hamburg Higher Education Law.

The **Faculty of Mathematics, Informatics and Natural Sciences (MIN)** in cooperation with the **Heinrich Pette Institute (HPI)** wishes to appoint at the earliest possible occasion a

W3 PROFESSORSHIP FOR STRUCTURAL BIOLOGY OF VIRUSES ref. 2145/W3

Tasks and responsibilities:

The successful candidate's future research should focus on the biology of human-pathogenic viruses. Experience in the investigation of infection processes using cryo-electron microscopy/electron tomography is essential. The group will maintain laboratories at HPI and at CSSB.

The Heinrich Pette Institute – Leibniz Institute for Experimental Virology is a research facility belonging to the Leibniz Gemeinschaft (WGL). HPI is associated with Universität Hamburg on the basis of a cooperative agreement. At HPI more than eighty scientists conduct research on human viruses with the aim of understanding viral diseases and developing new therapeutic approaches (www.hpi-hamburg.de)

The **Faculty of Mathematics, Informatics and Natural Sciences (MIN)** in cooperation with the **Bernhard Nocht Institute for Tropical Medicine (BNI)** wishes to appoint at the earliest possible occasion a

W3 PROFESSORSHIP FOR CELLULAR BIOLOGY OF HUMAN PARASITES

ref. 2146/W3

Tasks and responsibilities:

Candidates are expected to have a strong scientific record in the cellular biology of human parasites and to address topics of medical relevance and biological interest. Research activities should include approaches of systems biology designed to identify rate-limiting steps and decisive regulatory components of relevant metabolic pathways. The group will maintain laboratories at BNI and CSSB.

BNI is Germany's largest institution for research, medical services and training in the field of tropical diseases and emerging infections. The current research focus is on the molecular biology and immunology of plasmodia hemorrhagic fever viruses, mycobacteria and tissue nematodes. In cooperation with the Ghanaian Ministry of Health and Kumasi University, BNI runs a modern research and training centre in the West African rainforest, which is also available to external research groups (www.bnitm.de).

The **Faculty of Medicine at the University Medical Center Hamburg-Eppendorf (UKE)** wishes to appoint at the earliest possible occasion a

W3 PROFESSORSHIP FOR STRUCTURAL AND SYSTEMS BIOLOGY OF BACTERIA ref. FK 03-115/3

Tasks and responsibilities:

The candidate must demonstrate excellent scientific qualifications in the field of structural and/or systems biology of bacterial infectious agents and must describe in detail how he or she will use the various existing and planned radiation sources at DESY (Petra III, XFEL).

The University Medical Center Hamburg-Eppendorf has a research focus in the field of inflammation, infection and immunity.

Employment requirements:

Requirements for these posts are a university degree, a doctorate and additional scientific achievements via postdoctoral qualifications such as a habilitation, a junior professorship or scientific work at a university or research institution in accordance with § 15 of the Hamburg Higher Education law.

Applicants are expected to have international scientific experience. The scientific excellence of the applicant must be documented by publications in highly-ranked journals as well as by a successful track record in acquiring external research funding. The successful candidate is expected to develop CSSB into a first class research facility in the field of systems and structural biology, particularly with a focus on infectious diseases, in co-operation with the group leaders of the other CSSB units.

Post holders are required to teach four hours a week. Foreign applicants are expected to be able to teach in German in due time.

Severely disabled applicants will receive preference over equally qualified, non-disabled applicants.

Applications should include: a curriculum vitae, copies of references and certificates, a list of publications, details of teaching experience and proposals for future courses, a description of previous and planned research topics that make use of the radiation sources available at the DESY facilities, a proof of external funding acquired personally by the applicant.

Please send applications **for the professorship 2145/W3 or 2146/W3** in either paper or electronic (PDF) form to:

Den Präsidenten der Universität Hamburg, Referat Organisation & Personalentwicklung, Ausschreibungsstelle, Moorweidenstraße 18, 20148 Hamburg, Germany, or via email to: UniHHAusschreibungsstelle@verw.uni-hamburg.de.

Applicants for the professorship at the University Medical Center Hamburg-Eppendorf are asked to send four copies of the above documentation together with the **code number FK 03-115/3** to:

The Dean of the Faculty of Medicine, Universität Hamburg, Fakultäts-service -SV-, Martinstraße 52, 20246 Hamburg.

The deadline for applications is February 2nd, 2012.

For further information or enquiries, please contact Prof. Dr. Chris Meier (chris.meier@chemie.uni-hamburg.de) or Prof. Dr. Martin Aepfelbacher (m.aepfelbacher@uke.de).



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Faculty Positions in Science and Engineering

The Skolkovo Institute of Science and Technology
Moscow, Russia

The Skolkovo Institute of Science and Technology (SkTech) seeks candidates for tenured and tenure-track positions to begin July 1, 2012 or thereafter. SkTech is a new private university located just outside of Moscow and established in collaboration with the Massachusetts Institute of Technology (MIT).

SkTech aims to advance education, scholarship and economic development in the Russian Federation and beyond by educating leading graduate students and conducting research programs that address key challenges in science, technology and innovation.

The first group of faculty members will provide a model of inspired leadership in research, graduate education and innovation for others to follow and will have an opportunity to spend the first year in residence at MIT.

Applicants are invited to apply in SkTech's five areas of focus: Information Science and Technology, Biomedical Science and Technology, Energy Science and Technology, Space Science and Technology, and Nuclear Science and Technology.

Please visit <http://web.mit.edu/sktech> for more information and submit application materials to <https://sktech-search.mit.edu/> by January 31, 2012.

SkTech is committed to diversity and equity, and all are invited to apply without regard for gender, race or national origin. Compensation at SkTech is internationally competitive.

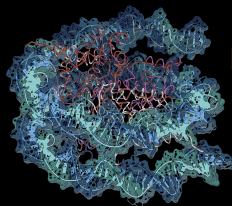


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Organisers:
Niall Dillon, Jesús Gil, Tim Aitman, Ana Pombo
www.csc.mrc.ac.uk/epigenreg2012
REGISTRATION DEADLINE: 18 MARCH 2012

Imperial College
London

THE UNIVERSITY OF HONG KONG



Founded in 1911, The University of Hong Kong is committed to the highest international standards of excellence in teaching and research, and has been at the international forefront of academic scholarship for many years. The University has a comprehensive range of study programmes and research disciplines spread across 10 faculties and about 100 sub-divisions of studies and learning. There are over 23,400 undergraduate and postgraduate students coming from 50 countries, and more than 1,200 members of academic and academic-related staff, many of whom are internationally renowned.

1. School of Biological Sciences

(A) Tenure-Track Professor in Food/Nutritional Sciences (Ref.: 201101146)

(B) Tenure-Track Associate Professor/Assistant Professor in Molecular Toxicology/Food Toxicology (Ref.: 201101147) **(C) Tenure-Track Associate Professor/Assistant Professor in Genomic/Proteomics/Metabolomics** (Ref.: 201101148) (from July 1, 2012 or as soon as possible thereafter. The positions are first offered on a three-year fixed-term basis, with the possibility of renewal and with consideration for tenure during the second fixed-term contract. For exceptionally outstanding candidates, early tenure may be considered.)

The School of Biological Sciences is ranked amongst the top five research schools/departments of life sciences in Asia (QS World University Rankings 2011). Current expertise of the School includes endocrinology, molecular biology, cell biology, biotechnology, ecology, aquatic biology, biodiversity, phylogenetics, environmental toxicology, food sciences and nutrition. Further information about the School can be obtained at <http://www.hku.hk/biosch/>.

For post (A), the appointee is expected to teach courses related to food sciences, food biotechnology and nutritional sciences. **For post (B)**, the appointee is expected to teach courses related to food toxicology, food safety and/or environmental toxicology. **For post (C)**, the appointee is expected to teach courses related to omics, molecular biology and cell biology.

For enquiries of the existing research activities and the specific job requirements, please write to Professor Rudolf Wu, Director of the School of Biological Sciences (e-mail: rudolfwu@hku.hk). Applicants should send a formal application, together with a curriculum vitae, a research plan, and a statement on teaching philosophy to sbsrec1@hku.hk.

Applicants who have responded to the previous advertisements (Ref.: 20100073 and 20100075) need not re-apply. **Closes March 31, 2012.**

2. Faculty of Science

Tenure-Track Associate Professor/Assistant Professor in Environmental Science (Ref.: 201101110) (from July 1, 2012 or as soon as possible thereafter. The post will initially be made on a three-year term with the possibility of renewal upon mutual agreement. Appointment with tenure will be considered during the second three-year contract.)

The Faculty of Science is expanding on its research and teaching program in environmental science. Information about the Faculty can be obtained at <http://www.hku.hk/science>.

For an Associate Professorship, applicants should be able to demonstrate excellence in teaching and research in related areas and should possess a proven record of high-quality scholarship and services. The appointee should be able to provide academic leadership in both research and teaching, and is also expected to supervise postgraduate students, conduct an ongoing active research agenda, obtain research grants, and serve on committees within the University and in external professional organizations. **For an Assistant Professorship**, applicants should possess good teaching and research skills and be able to demonstrate a strong potential for research excellence. The appointee is expected to commit to high-quality scholarly research, submit grant proposals, publish in top-tier academic journals, and supervise postgraduate students. **For enquiries of the existing research activities and the specific job requirements, please write to Professor Sun Kwok, Dean of Science** (e-mail: deansci@hku.hk). Applicants should send a completed application form, together with a curriculum vitae, a detailed publication list, a research plan and a statement on teaching philosophy, which includes a portfolio of syllabi and descriptions of courses they have taught or co-taught, to sciappt@hku.hk. They should also arrange for submission of three references from senior academics who are familiar with their teaching approaches, skills and experience. **Closes January 31, 2012.**

Applicants should indicate clearly the reference number in the subject of the e-mail and which level they wish to be considered for. The appointee is expected to develop an active research programme, publish in top-tier journals, and contribute to both undergraduate teaching and graduate supervision. Applicants for a Professorship/Associate Professorship should have a strong record of obtaining external grant support, and are expected to provide academic leadership in their research areas. Applicants for an Assistant Professorship should demonstrate a strong potential for obtaining competitive external funding and conducting high-quality research.

A globally competitive remuneration package commensurate with the appointee's qualifications and experience will be offered. The appointments will attract a contract-end gratuity and University contribution to a retirement benefits scheme, totalling up to 15% of basic salary, as well as leave, and medical benefits. Housing benefits will be provided as applicable. At current rates, salaries tax does not exceed 15% of gross income.

Application form (341/1111) can be obtained at <http://www.hku.hk/apptunit/form-ext.doc>. Further particulars can be obtained at <http://www.hku.hk/apptunit/>. The University thanks applicants for their interest, but advises that only shortlisted candidates will be notified of the application result.

The University is an equal opportunity employer and is committed to a No-Smoking Policy

POSITIONS OPEN

FACULTY POSITION Molecular Cell Biology

The Department of Biological Sciences at Rutgers University–Newark invites applications for a state-funded, tenure-track **ASSISTANT PROFESSOR** position. Applicants must have a Ph.D. degree or equivalent and postdoctoral experience along with a track record of research accomplishment. We are searching for a faculty colleague working in the broadly defined area of Molecular Cell Biology. Applicants whose research program examines cytoskeleton, cell polarity, cell-cell interaction, organelle biogenesis, or signal transduction using multiple methodologies including molecular, genetic, and live cell imaging on vivo and/or in vitro systems are strongly encouraged to apply.

The successful candidate will receive generous start-up support and ample laboratory space in anticipation of developing a robust, externally funded, independent research program. The candidate will also contribute to the undergraduate and graduate teaching missions of the department.

Applicants should submit a single PDF document containing a cover letter, curriculum vitae, brief summary of current and proposed research plans, selected publications and have three letters of recommendations sent to e-mail: biosarc@newark.rutgers.edu. Review of applications will begin January 20, 2012 with an anticipated start date of September 2012. *Rutgers University is the recipient of the NSF ADVANCE Institutional Transformation Award and it is also an Affirmative Action/Equal Opportunity Employer.*

THE SARAH AND DANIEL HRDY Fellowship in Conservation Biology Department of Organismic and Evolutionary Biology Harvard University

The Department of Organismic and Evolutionary Biology invites applications or nominations for the Sarah and Daniel Hrdy Visiting Fellowship in Conservation Biology. The Hrdy Visiting Fellowship is available either at the senior faculty level or at the junior (i.e., postdoctoral) level for one or two semesters. Duties include teaching one course and/or giving lectures in conservation biology, as well as research and collaboration with members of the Harvard community. Recipients of this fellowship are expected to have a strong and transformative effect on the study of conservation biology at Harvard, from the undergraduate to the senior teaching level. The fellowship includes a stipend with modest additional funds for research and teaching. Applicants should contact a faculty sponsor(s), with whom they will collaborate, before applying. Applications should include a cover letter with a statement of intent, curriculum vitae, and representative publications, and applicants should arrange to have three letters of reference sent.

Please submit applications online at **website:** <http://academicpositions.harvard.edu/postings/3911>.

Applicants should contact a faculty sponsor(s), with whom they will collaborate, before applying. Applications should include a cover letter with a statement of intent, curriculum vitae, and representative publications, and applicants should arrange to have three letters of reference sent.

Further information about OEB is available at **website:** <http://www.oeb.harvard.edu>. Address questions about the application/nomination process to **Mr. Christopher Preheim** in OEB e-mail: cpreheim@oeb.harvard.edu.

Review of applications will begin on March 1, 2012.

Harvard University is an Affirmative Action/Equal Opportunity Employer. Applications from women and minority candidates are strongly encouraged.

POSITIONS OPEN

BIOLOGY POSITIONS University of Wisconsin–Parkside

The Biological Sciences Department at the University of Wisconsin–Parkside invites applications for two positions, both beginning August 2012. First, a Tenure-Track **ASSISTANT PROFESSOR** (nine month) position in Mammalian Physiology, primary responsibilities: teaching, research, and service. Qualifications include: Ph.D., postdoctoral experience, relevant research and teaching experience, and sensitivity to, or experience in, working with a diverse, multicultural population. Second, a **LECTURER** (nine month) position in Human Anatomy, primary responsibility for teaching. Qualifications include: M.S. or Ph.D., relevant teaching experience, and sensitivity to, or experience in, working with a diverse, multicultural population. Applications for both positions received by February 15, 2012 are ensured full consideration; positions are open until filled. For more details visit **website:** <http://www.uwp.edu/departments/human.resources/unclassified.positions/index.cfm>. *University of Wisconsin–Parkside is an Affirmative Action/Equal Employment Opportunity Employer.*

ASSOCIATE RESEARCH SCIENTIST Department of Neurological Surgery Columbia University

Columbia University's Department of Neurological Surgery invites applications for two research positions available to begin January 2012. Ph.D. and/or M.D. and a strong research background in either spinal neuronal networks or the cellular mechanisms of glioblastoma are required.

Both positions require a wide range of technical skills, including confocal microscopy, electron microscopy, single-cell microdissection, RNA and DNA amplification, and fluorescent labeling. The successful applicant will also have expertise in developing transgenic mouse models.

All applications must be made through Columbia University's online RAPS application system at **website:** <https://academicjobs.columbia.edu/applicants/Central?quickFind=55680>.

Columbia University is an Equal Opportunity/Affirmative Action Employer.

POSTDOCTORAL POSITION in Brain Targeting/Cancer Immunology

A position (two years) is available immediately in the laboratory of **Ulrich Bickel, M.D.**, Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center at Amarillo. Our group focuses on blood-barrier transport mechanisms and drug delivery. The successful applicant will work on an innovative new project exploring the targeting of breast cancer metastasis in brain using a novel type of monoclonal antibody (*J. Cell Physiol.* **225**:664–672; *J. Immunol.* **186**: 3265–3276) funded by the Cancer Prevention and Research Institute of Texas (CPRIT). Background in pharmacology/pharmaceutical sciences or related areas with experience in pharmacokinetic studies in rodents, cell culture, and histological techniques, including (confocal) fluorescence microscopy is advantageous. For more information about this position and to apply, visit our **website:** <http://jobs.texasstate.edu>. *Equal Opportunity Employer/Affirmative Action/ADA.*

ASSISTANT/ASSOCIATE/ FULL PROFESSOR Biomedical Research and Technology

The College of Sciences at the University of Nevada, Las Vegas seeks applicants for a tenure-track or tenured faculty position in the broad disciplinary area of Biomedical Research and Technology. This faculty position may reside in the School of Life Sciences, or in other departments within the College of Sciences, as appropriate. A complete job description with application details may be obtained by visiting **website:** <http://jobs.unlv.edu> or calling **telephone:** 702-895-2894. *Equal Employment Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

DIRECTOR

U.S. Geological Survey Western Fisheries Research Center, Seattle. Molecular to ecosystem scale fisheries research throughout the Western U.S.; six laboratory locations support genetic, conservation, habitat, climate change, and other investigations. Competitive salary and federal benefits. Apply at **website:** <http://www.usajobs.gov>. Title: Western Fisheries Research Center Director, GS-0401/0482-15. *The federal government is an Equal Opportunity Employer.*

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4-1BB Receptor	Caspase-6	sFlt-1 (D4)	IL-3	Mek-1	sRANKL
6 Ckine	CD4	sFlt-1 (D5)	IL-4	MIA	RANTES
ACAD8	CD14	sFlt-1 (D7)	sIL-4 Receptor	Midkine	RELM- α
ACAT2	CD22	Flt3-Ligand	IL-5	MIG / CXCL9	RELM- β
gAcrp30/Adipolean	CD40 Ligand / TRAP	sFlt-4	IL-6	MIP-1 α / CCL3	Resistin
Activin A	CD95 / sFas Ligand	sFlt-4/ Fc Chimera	sIL-6 Receptor	MIP-1 β / CCL4	RPTP β
ACY1	CD105 / Endoglin	Follistatin	IL-7	MIP-3 / CCL23	RPTP γ
ADAT1	CHIPS	FSH	IL-8 (72 a.a.)	MIP-3 α / CCL20	RPTP μ
Adiponectin	CNTF	Fractalkine/ CX3C	IL-8 (77 a.a.)	MIP-3 β / CCL19	SCF
ADRP	Collagen	G-CSF	IL-9	MIP-4 (PARC) / CCL18	SCGF- α
AITRL	CREB	α -Galactosidase A	IL-10	MIP-5 / CCL15	SCGF- β
Akt1	CTACK/CCL27	Galectin-1	IL-11	MMP-3	SDF-1 α
Alpha-Feto Protein (AFP)	CTGF	Galectin-3	IL-12	MMP-7	SDF-1 β
Alpha-Galactosidase A	CTGFL/WISP-2	Gastrointestinal CA	IL-13	MMP-13	Secretin
Angiopoietin-1 (Ang-1)	CTLA-4/Fc	GCP-2	IL-13 analog	Myostatin	SF20
Angiopoietin-2 (Ang-2)	CXCL16	GDF-3	IL-15	Nanog	SHP-2
Angiostatin K1-3	Cytokeratin 8	GDF-9	IL-16 (121 a.a.)	NAP-2	STAT1
Annexin-V	DEP-1	GDF-11	IL-16 (130 a.a.)	Neurturin	c-Src
apo-SAA	Desmopressin	GDNF	IL-17	NFAT-1	TACI
Apolipoprotein A-1	Disulfide Oxidoreductase	GLP-1	IL-17B	beta-NGF	TARC
Apolipoprotein E2	E-selectin	Glucagon	IL-17D	NOGGIN	TC-PTP
Apolipoprotein E3	ECGF	Goserelin	IL-17E	NOV	TECK
Apolipoprotein E4	EGF	GM-CSF	IL-17F	NP-1	TFF2
APRIL	Elafin/SKALP	GPBB	IL-19	NT-1/BCSF-3	TGF- α
Artemin	EMAP-II	GRO α	IL-20	NT-3	TGF- β 1
ATF2	ENA-78	GRO β	IL-22	NT-4	TGF- β 2
Aurora A	Endostatin	GRO γ	IL-31	Ocreotide	TGF- β 3
Aurora B	Enteropeptidase	GRO/MGSA	Insulin	Oncostatin M	Thymosin α 1
BAFF	Eotaxin	Growth Hormone	IP-10	Osteoprotegerin (OPG)	sTIE-1/Fc Chimera
BAFF Receptor	Eotaxin-2	Growth Hormone BP	JE	OTOR	sTIE-2/Fc Chimera
BCA-1 / BLC / CXCL13	Eotaxin-3 (TSC)	GST-p21/WAF-1	JNK2a1	Oxytocin	TL-1A
BCMA	EPHB2	HB-EGF	JNK2a2	p38- α	TNF- α
BD-1	EPHB4	HCC-1	KC / CXCL1	Parathyroid Hormone	TNF- β
BD-2	Eptifibatide	HGF	KGF	PDGF-AA	sTNFR1
BD-3	Erk-2	Histidyl-tRNA synthetase	L-asparaginase	PDGF-AB	sTNFR2
BDNF	Erythropoietin (EPO)	Histrelin	LAG-1	PDGF-BB	TPO
Bivalirudin	Exodus-2	HRG1- β 1	LALF Peptide	Persephin	TRAIL/Apo2L
BMP-2	Fas Ligand	I-309	LAR-PTP	PF-4	sTRAIL R-1 (DR4)
BMP-4	Fas Receptor	I-TAC	LC-1	PIGF-1	sTRAIL R-2 (DR5)
BMP-7	FGF-1 (acidic)	IFN- α	LBP	PIGF-2	TSH
BMP-13	FGF-2 (basic)	IFN- α A	LD-78 β	PKA α -subunit	TSLP
sBMPR-1A	FGF-4	IFN- α 2a	LDH	PKC- α	TWEAK
Brain Natriuretic Protein	FGF-5	IFN- α 2b	LEC/NCC-4	PKC- γ	TWEAK Receptor
BRAK	FGF-6	IFN- β	Leptin	Pleiotrophin	Urokinase
Breast Tumor Antigen	FGF-7/ KGF	IFN- γ	LIGHT	PLGF-1	VEGF121
C5a	FGF-8	IFN-Omega	LIX	Polymyxin B (PMB)	VEGF145
C5L2 Peptide	FGF-9	IGF-I	LKM	PRAS40	VEGF165
C-10	FGF-10	IGF-II	LL-37	PRL-1	VEGF-C
C-Reactive Protein	FGF-16	proIGF-II	Lymphotactin	PRL-2	VEGF-C I525
C-Src	FGF-17	IGFBP-1	sLYVE-1	PRL-3	EG-VEGF
Calbindin D-9K	FGF-18	IGFBP-2	M-CSF	Prokineticin-2	VEGF-E
Calbindin D-28K	FGF-19	IGFBP-3	MCP-1 (MCAF)	Prolactin	HB-VEGF-E
Calbindin D-29K	FGF-20	IGFBP-4	MCP-2	Protirelin	sVEGFR-1
Calmodulin	sFGFR-1 (IIIc) / Fc Chimera	IGFBP-4	MCP-3	PTHrP	sVEGFR-2
Calcitonin Acetate	sFGFR-2 (IIIc) / Fc Chimera	IGFBP-5	MCP-4	PTP1B	sVEGFR-3
Carbonic Anhydrase III	sFGFR-3 / Fc Chimera	IGFBP-6	MCP-5	PTP-IA2	WISP-1
Carcino-embryonic Antigen	sFGFR-4 / Fc Chimera	IGFBP-7	MDC (67 a.a.)	PTP-MEG2	WISP-2
Cardiotrophin-1	sFlt-1 (native)	IL-1 α	MDC (69 a.a.)	PTP-PEST	WISP-3
		IL-1 β	MDH		WNT-1



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Essay: CDK Directs the
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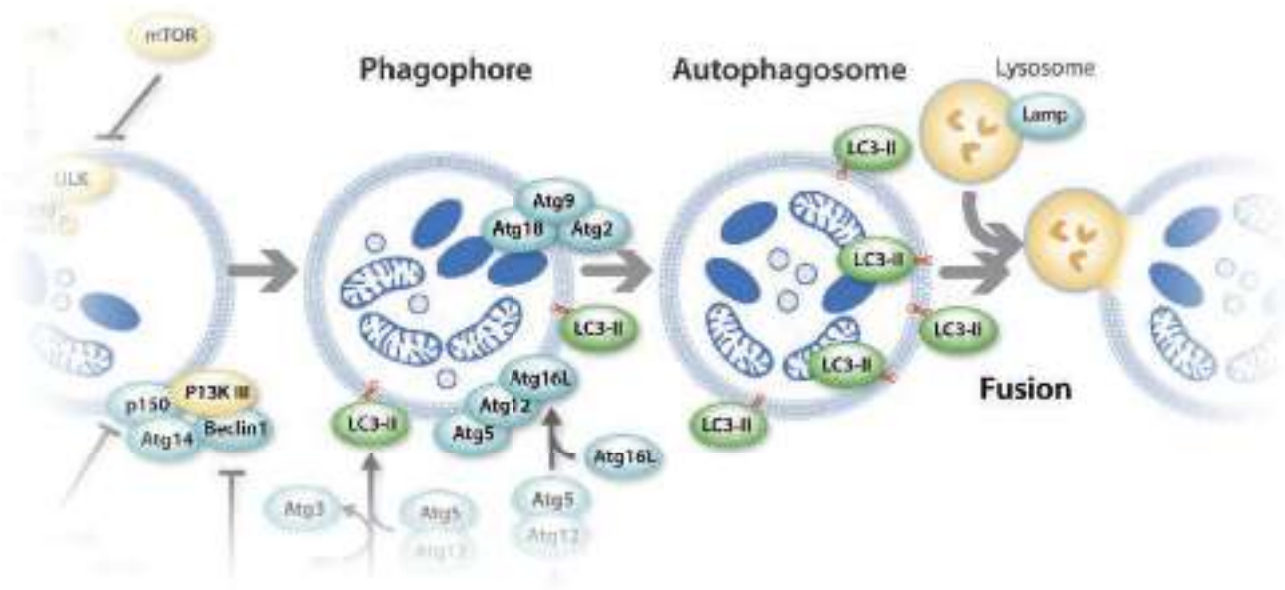
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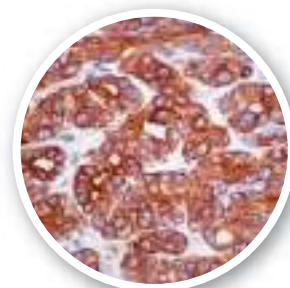
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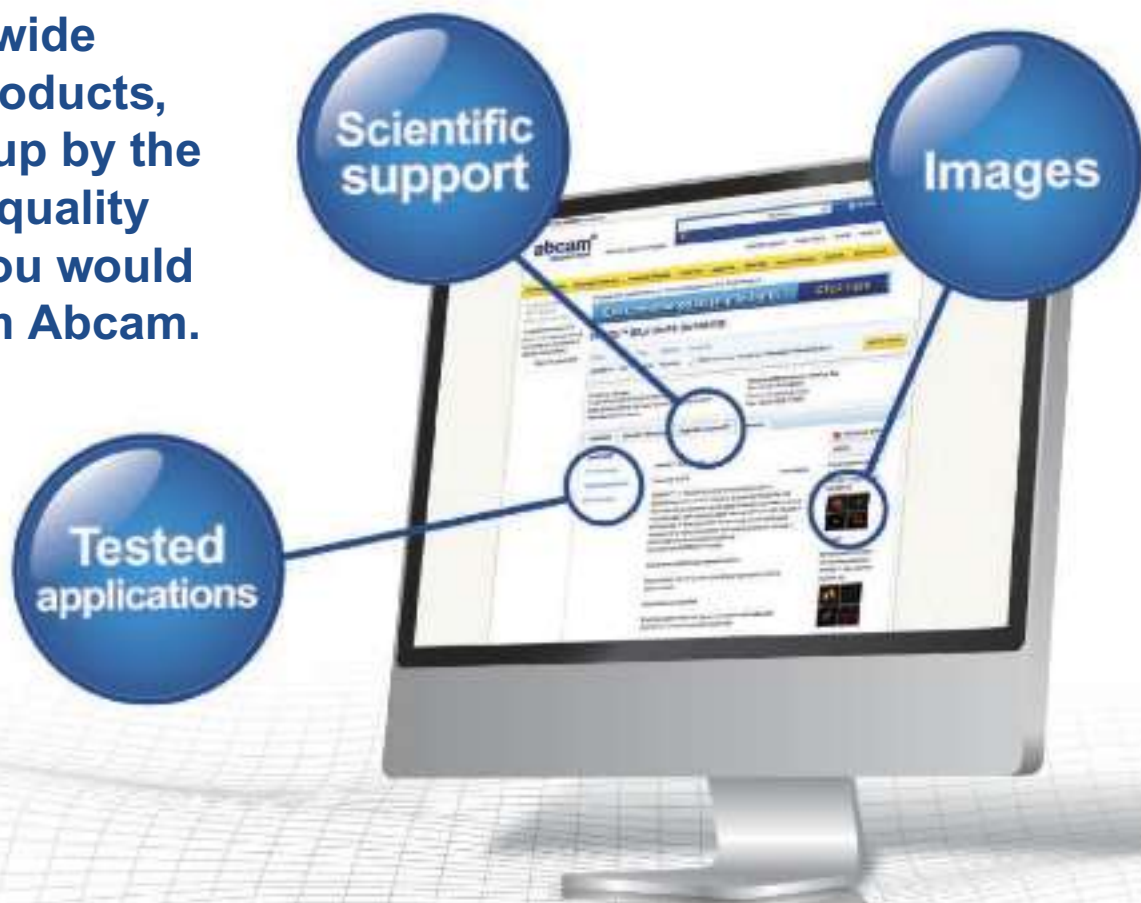
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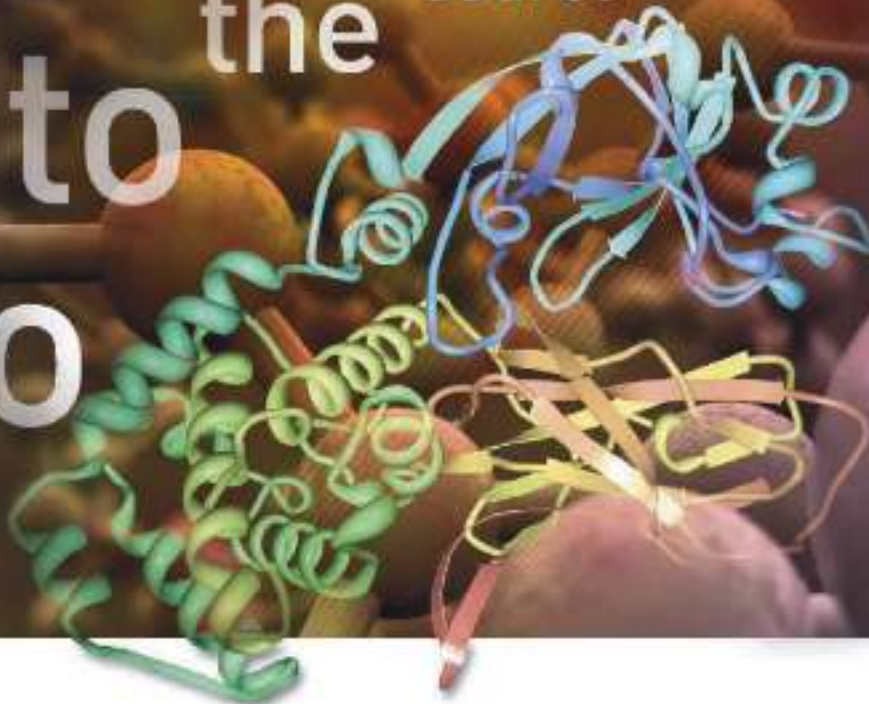
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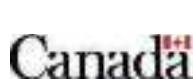


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